

the Acid Deposition Research Program

BIOPHYSICAL RESEARCH

**CRITICAL REVIEW OF INORGANIC
SULPHUR MICROBIOLOGY WITH
PARTICULAR REFERENCE TO
ALBERTA SOILS**

by

E.J. LAISHLEY and R. BRYANT

Department of Biology
The University of Calgary
Calgary, Alberta, Canada

February, 1987

PRIME RESEARCH CONTRACTOR:

The Kananaskis Centre for Environmental Research
The University of Calgary
Calgary, Alberta, Canada





Digitized by the Internet Archive
in 2015

<https://archive.org/details/criticalreviewof00lais>

The Acid Deposition Research Program is funded and administered by the Province of Alberta, the Canadian Petroleum Association, Alberta's electrical utilities and the Energy Resources Conservation Board.

A distinctive feature of the ADRP is the development and funding of research in two major areas, biophysical and human health.

Acid Deposition Research Program - Members Committee

Ron L. Findlay (Co-Chairman)
Manager, Environmental Affairs
AMOCO Canada Petroleum Co. Ltd.

E. Millard Wright
Manager, Environmental Planning
Gulf Canada Corporation

Carl L. Primus (Co-Chairman)
Assistant Deputy Minister
Alberta Environment

Ed Brushett
Manager, Environmental Protection
Energy Resources Conservation Board

Dr. John Raiton
Manager, Environmental Planning
TransAlta Utilities

Dr. Brian O'Connor
Director of Health Promotion and
Protection Programs
Alberta Community and Occupational Health

Dr. Harby S. Sandhu
Senior Research Manager
Research Management Division
Alberta Environment

Dr. Martha Kostuch
Public Representative

Ken Smith
Assistant Deputy Minister
Alberta Environment

Program Manager: **Dr. Ron R. Wallace**
Communications Co-ordinator: **Jean L. Andryiszyn**

Scientific Advisory Board

Biophysical Research

Dr. Sagar V. Krupa
University of Minnesota
(Department of Plant Pathology)

Dr. T. Craig Weidensaul
Ohio Agricultural Research
and Development Center
Ohio State University

Dr. James P. Lodge
Consultant in Atmospheric Chemistry
and Editor, Atmospheric Environment

Dr. Douglas P. Ormrod
University of Guelph
Department of Horticultural Science

Dr. Robert K. Stevens
U.S. Environmental Protection Agency

Dr. Carl L. Schofield
Cornell University
Department of Natural Resources

Dr. C. M. Bhumralkar
National Oceanographic
and Atmospheric Administration (NOAA)

Dr. Ron Kickert
Consultant (in modelling)

Bruce B. Hicks
National Oceanographic
and Atmospheric Administration
Atmospheric Turbulence Diffusion
Laboratory (NOAA)

Dr. Herbert C. Jones
Tennessee Valley Authority
Fisheries and Aquatic Ecology Branch

Dr. M. Ali Tabatabai
Iowa State University
Department of Agronomy

Biophysical Research Prime Contractor:
Kanaskis Centre for Environmental Research
The University of Calgary

Principal Investigator: **Dr. Allan H. Legge**

Acid Deposition Research Program, 1500, 633 Sixth Avenue S.W., Calgary, Alberta, Canada, T2P 2Y5

CRITICAL REVIEW OF INORGANIC SULPHUR MICROBIOLOGY
WITH PARTICULAR REFERENCE TO ALBERTA SOILS

by:

E.J. Laishley and R. Bryant

Department of Biology
The University of Calgary
Calgary, Alberta, Canada

prepared for:

Kananaskis Centre for Environmental Research
The University of Calgary
Calgary, Alberta, Canada

for submission to:

The Acid Deposition Research Program
1500, 633 Sixth Avenue S.W.
Calgary, Alberta, Canada, T2P 2Y5

February 1987

This publication may be cited as:

Laishley E.J. and Bryant R., 1987.

Critical Review of Inorganic Sulphur Microbiology with Particular Reference to Alberta Soils. Prep for the Acid Deposition Research Program by the Department of Biology, The University of Calgary. ADRP-B-04/87. 56 pp.

ISBN 0-921625-06-5 (Volume 4)

ISBN 0-921625-02-2 (Set of 11)

EXECUTIVE SUMMARY

1. The physiology and biochemistry of the microorganisms participating in the sulphur cycle are discussed.
2. Of particular importance to the Alberta situation are the sulphur oxidizing microorganisms belonging to the "Colourless Sulphur Bacteria" group, with special reference to the thiobacilli.
3. New species of the thiobacilli native to Alberta have been isolated and characterized; their biological properties are compared to other known species.
4. Biological oxidation of inorganic sulphur can have economic and ecological repercussions (i.e., (a) creation of acid soils, with accompanying leaching of nutrients such as Fe^{2+} and Al^{3+} resulting in toxicity to plants, and (b) acid production causing direct damage to plant tissues).
5. Three main factors affecting the oxidation of fugitive (wind blown) sulphur include the sulphur itself, the sulphur oxidizing microorganisms, and the soil environment.
6. With regard to the sulphur itself, its microcrystalline structure and particle size affect the biological oxidation rate.
7. Different sulphur microorganisms can oxidize sulphur at different rates; the most prolific oxidizers belong to the genus Thiobacillus.
8. The microbial oxidation of sulphur in the soil is markedly affected by temperature, moisture and nutrients, and soil type.
9. Fugitive (wind blown) sulphur from existing stock piles or prilling operations will create an ideal soil environment for the sulphur oxidizing thiobacilli, the end result being acidification of the soil.
10. Long term studies (eight years - see Lore (1984) in References Cited) of SO_2 contamination of soils from a nearby gas plant did not show acidification trends, suggesting that acidification of soils from SO_2 deposition is not as severe as wind blown sulphur dust contamination.
11. Liming an acid soil polluted with sulphur only masks an existing problem and really provides a more favourable environment for continued microbial sulphur oxidation and acidification.

12. More extensive microbiological research on liming acid soils and the subsequent effects on soil properties and dynamics is required.
13. To prevent the thiobacilli from oxidizing fugitive sulphur, the following steps are recommended: (a) reduce the sulphur content in the soil; (b) develop a new liming procedure; and, (c) stop the fugitive sulphur at the source.
14. A comparative time study on the effectiveness of CaCO_3 versus Ca(OH)_2 in treating acid soils, and on the effect of these treatments on the thiobacilli and general soil microbial activity is essential.
15. The effect of liming an already acid polluted soil on the physiological groups of microorganisms needs to be studied to ascertain the time required for re-establishment of normal soil biological activity.
16. More studies on the mechanisms of the adhesion of the thiobacilli to sulphur are required so that we are in a better position to make recommendations regarding control of these organisms and their acidification process in soil.
17. We have pioneered the TEW (Thiobacillus Early Warning) system which monitors and predicts in advance those soils which may become acid due to fugitive sulphur contamination.

TABLE OF CONTENTS

	Page
EXECUTIVE SUMMARY	i
TABLE OF CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	iv
ACKNOWLEDGEMENTS	v
1. INTRODUCTION	1
2. THE SULPHUR CYCLE - MICROBIOLOGY AND BIOCHEMISTRY	3
2.1 Background	3
2.1.1 Oxidation Reactions	5
2.1.1.1 Colourless Sulphur Bacteria	5
2.1.1.1.1 Genus <u>Thiobacillus</u>	7
2.1.1.1.2 Genera <u>Sulfolobus</u> , <u>Thiomicrospira</u> and <u>Thermothrix</u>	14
2.1.1.1.3 Genus <u>Beggiatoa</u>	15
2.1.1.2 Phototrophic Sulphur Bacteria: Anaerobic Oxidation of Reduced Sulphur Compounds	15
2.1.1.3 Heterotrophic Microorganisms	17
2.1.2 Reduction Reactions	18
3. ECOLOGICAL AND ECONOMIC ACTIVITIES OF MICROBIAL INORGANIC SULPHUR OXIDATION AND REDUCTION	21
3.1 Oxidation Reactions	21
3.1.1 The Colourless Bacteria and Heterotrophs	21
3.1.1.1 Oxidation of Sulphur	21
3.1.1.2 Oxidation of Metal Sulphides in Soil	23
3.1.2 The Phototrophic Sulphur Bacteria	25
3.2 Reduction Reactions	25
4. FACTORS AFFECTING THE MICROBIAL OXIDATION OF SULPHUR	27
4.1 Sulphur	27
4.1.1 Composition	27
4.1.2 Particle Size and Weight	28
4.2 Sulphur Oxidizing Microorganisms	28
4.3 Soil Environment	29
4.3.1 Temperature	29
4.3.2 Soil Type	30
4.3.3 Moisture and Nutrients	31
5. THE ALBERTA PROBLEM	33
6. PREVENTION AND RECLAMATION OF ACID SOILS	39
6.1 Acid Soils - Reclamation	39
6.2 Acid Soils - Sulphur Pollution, A Special Case	40
7. FUTURE WORK	41
8. REFERENCES CITED	43

LIST OF TABLES

	Page
1. The Colourless Sulphur Bacteria	6
2. Chemical Reactions of the Thiobacilli	9
3. Characteristics of Less Acidophilic Thiobacilli	10
4. Characteristics of Acidophilic Thiobacilli	13
5. Average Crop Yield as Influenced by Soil pH and Liming	36

LIST OF FIGURES

	Page
1. Global Sulphur Cycle	4
2. Ways in Which Inorganic Sulphur Compounds May Be Oxidized to SO_4^{-2}	8
3. Photosystem in Green and Purple Bacteria	16
4. Oxidation of Lactic Acid and the Dissimilatory Reduction of Sulphate by <u>Desulfovibrio</u> sp	19
5. Direct and Indirect Oxidation Mechanisms for Pyrite Oxidation	24
6. Location of Soil Testing Areas in Alberta, and the Percentage of Cultivated Soil with a pH of 6.0 or Less for Each Area	34
7. Soils of Alberta	35

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Alberta Government/Industry Acid Deposition Research program for providing funding for this research. The typing of the manuscript by Lynn Ewing of the Kananaskis Centre for Environmental Research, The University of Calgary, is also appreciated.

1. INTRODUCTION

Until recently sulphur in the soil system has not received a great deal of attention. A renewed interest in this subject is due in part to the sulphur deficiencies in various agricultural soils throughout the world (Coleman 1966; Wainwright 1978; Beaton and Soper 1985) and also because one of the attributed causes of the spreading acid soil phenomenon has been the photo- and wet chemical oxidation of fugitive sulphur and nitrogen oxides present in industrial emissions. The oxides are converted to their corresponding acidic anions and are deposited onto the soils during rain (Cadle 1975; Takahashi et al. 1975; Babich and Stotzky 1978a; and Grennfelt et al. 1980). More specifically, Kellogg et al. (1972) estimated that 95% of anthropogenic emissions of atmospheric sulphur are in the form of SO₂ (sulphur dioxide). As Alberta is the world's largest producer of sulphur, the potential for acid deposition problems from industrial processing (SO₂ from sour gas processing or sulphur dust from S⁰ granulation operations) and storage of sulphur are real and cannot be overlooked. The problem of acidification of soils is much more complex, however, as there are other natural processes by which soils may become acidified. These are summarized as follows:

1. acid production from carbonation of water (Ulrich 1980; Tabatabai 1985);
2. acid production from the microbial decomposition of plant residues (Stevenson 1967; Tabatabai 1985);
3. acid production from microbial nitrification (Alexander 1971);
4. acid production from the oxidation of sulphide minerals (Alexander 1971; Metson et al. 1977);
5. acid production from the microbial oxidation of elemental S (Attoe and Olson 1966; Adamczyk-Winiarska et al. 1975; Bollen 1977; and Laishley and Bryant 1985);
6. acid production from the microbial oxidation of sulphur construction materials (Laishley et al. 1979; Laishley and Bryant 1985).

It is apparent from this list (as in Laishley (1985)) that microorganisms play a key role in the transformation of sulphur compounds to acid. The same microorganisms are also involved in maintaining the intricate sulphur cycle which is critical to soil fertility. Part of the purpose of this review is to examine the sulphur cycle and gain an understanding of the beneficial and detrimental roles performed by various sulphur microorganisms. As well, one should come to learn that in certain ecosystems the balance of the cycle has shifted (sometimes naturally and sometimes artificially) and that these shifts can have ecological and economic implications.

2. THE SULPHUR CYCLE - MICROBIOLOGY AND BIOCHEMISTRY

2.1 BACKGROUND

Sulphur in the form of sulphate ion (SO_4^{2-}) is required by higher plants for the synthesis of sulphur-containing amino acids and ultimately for protein synthesis. The problem is that most of the sulphur present in soils is in the unavailable organic sulphur compound form, particularly in humid regions. For example, Freney (1961) stated that a value of 93% organic sulphur was typical of 24 Australian soils. High organic sulphur content values (42 - 77%) have also been reported in soils from various parts of the world (Walker and Adams 1958; Tabatabai and Bremner 1972). It is therefore important that these organic sulphur compounds be transformed to SO_4^{2-} form which subsequently would be available for plant uptake.

Breakdown of organic sulphur is essentially accomplished in two states: mineralization of organic compounds and transformation of the reduced inorganic sulphur to SO_4^{2-} . The first process, mineralization of organic compounds, refers to the release of inorganic S (reduced and oxidized) from organic S and is accelerated by a wide variety of heterotrophic microorganisms which derive their energy from the decomposition of organic matter. If the sulphur content of the substrate is greater than that which can be utilized in biosynthesis by the microorganisms, the excess is made available for other soil processes (Reuss 1975). It is true that such microbial decomposition produces both organic and inorganic sulphur compounds and in these degradation reactions bacteria (e.g., Escherichia sp., Bacillus sp., Pseudomonas sp., Proteus sp., Sarcina sp., and Clostridium sp.), actinomycetes (e.g., Streptomyces sp.), and fungi (e.g., Microspora sp., Aspergillus sp., Candida sp., Fusarium sp., Microsporium sp., Scopuluriopsis sp., and Oidium sp.) have been shown to produce a host of volatile organic compounds such as methyl mercaptan, dimethyl sulphide, dipropyl sulphide, and carbonyl disulphide (Babich and Stotzky 1978a) as well as non-volatile organic compounds such as the amino acids methionine and cysteine. Although these compounds may exert both negative effects (inhibition of nitrification by CS_2) and positive effects (e.g., dimethyl sulfide provides broccoli with resistance to infection by fungus Peronospora parasitica) on the soil system (Powelson and Jenkinson 1971; Bremner and Bundy 1974; and Greenhalgh and Mitchell 1976)), very little is known about the overall impact of organic sulphur compounds on soil properties and dynamics (Babich and Stotzky 1978a). However, the majority of organic sulphur is released as inorganic substances like sulphide, elemental sulphur (S^0), thiosulphate ($\text{S}_2\text{O}_3^{2-}$), and tetrathionate ($\text{S}_4\text{O}_6^{2-}$) (Starkey 1966).

The potential for environmental problems from atmospheric sulphur compounds in Alberta is largely due to inorganic sulphur compounds (particularly S^0 and SO_2) emanating from anthropogenic activities. This review will, therefore, concentrate on the microbial transformation of inorganic sulphur compounds and the sulphur bacteria which are responsible for the transformation of most of the inorganic sulphur in soil (Starkey 1966). A simplified sulphur cycle is presented in Figure 1. It will be immediately observed that SO_2 is generally not included as part of the cycle. There are reasons for this and some comments regarding SO_2 are in order. SO_2 is highly water soluble (11.28 g/100 ml at 20°C) and will, upon contact with H_2O (atmospheric or soil), abiotically oxidize to sulphurous acid (H_2SO_3) (Babich and Stotzky 1978b). Moreover, depending upon the

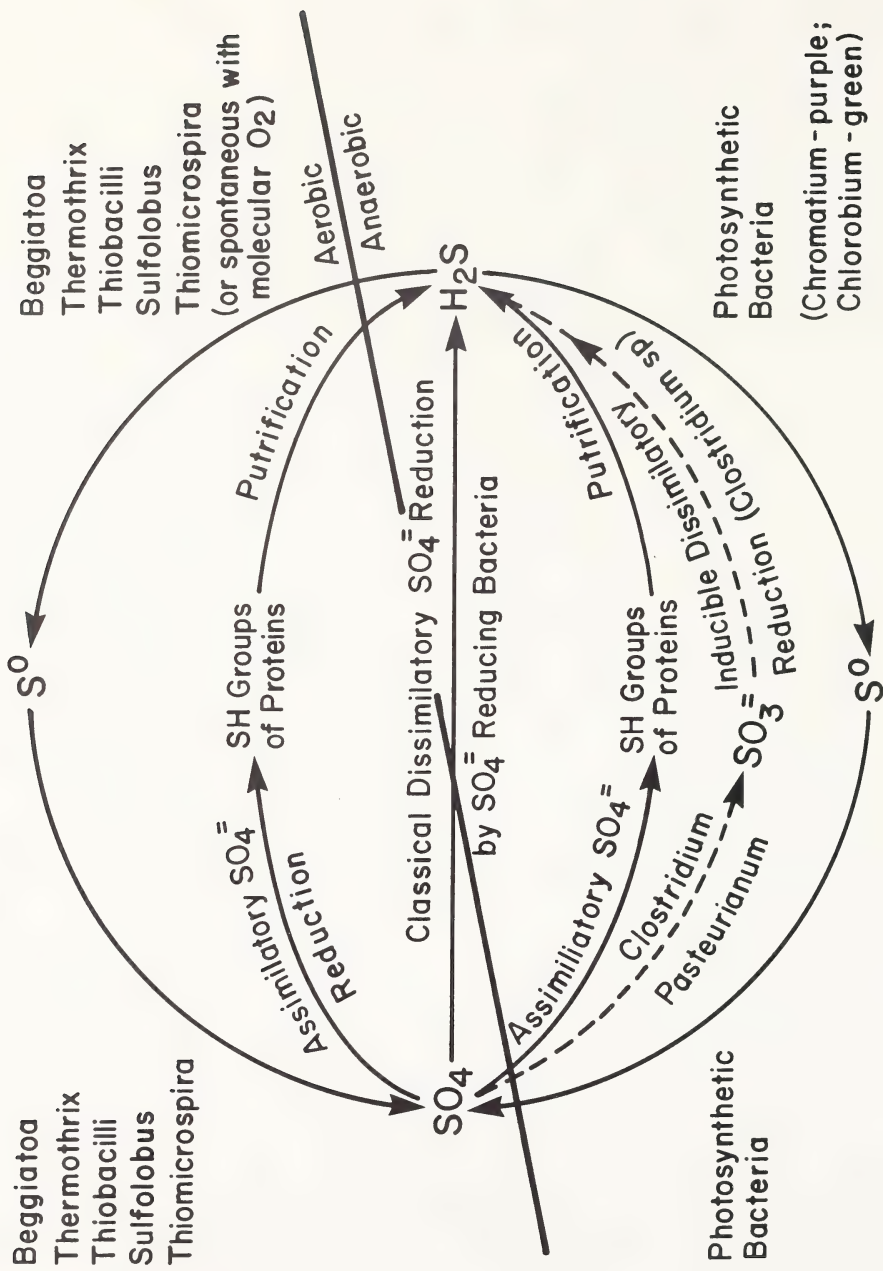
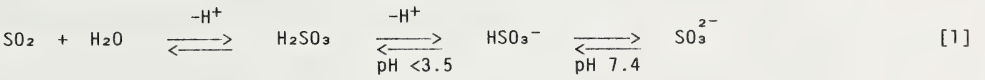


Figure 1. Global sulphur cycle. (Modified after Brock et al. 1984; Laishley 1978; Laishley et al. 1984)

environmental pH, H₂SO₃ exists in an equilibrium with bisulphite (HSO₃⁻) and sulphite (SO₃²⁻), best described by the following reactions (Babich and Stotzky 1978b; Gunnison 1981):



Unless strong acid conditions (i.e., pH<3.5) already prevail, much of the SO₂ will be in an equilibrium with HSO₃⁻ and SO₃²⁻. However, as HSO₃⁻ and SO₃²⁻ are known to be very labile species which rapidly oxidize (mostly aerobically and abiotically) to SO₄²⁻, it seems that much of the SO₂ released in the atmosphere will end up in non-acidic soil as SO₄²⁻ (Babich and Stotzky 1978a; Wainwright 1980). Although certain reports suggest that the soil microflora may be involved in some small capacity in the oxidation of SO₂ and SO₃²⁻ to SO₄²⁻ (Ghiorse and Alexander 1976; Wainwright 1980), evidence suggests that the majority of SO₂ quickly oxidizes to SO₄²⁻ abiotically and as such SO₂ is excluded from Figure 1. The sulphur cycle presented here, which involves microorganisms as catalysts for the reactions, will be considered in further detail from two perspectives, namely oxidation and reduction reactions.

2.1.1 Oxidation Reactions

Inorganic sulphur oxidation and reduction reactions can occur under aerobic and anaerobic conditions, in the absence and presence of light (Jorgensen 1982). In considering the oxidation reactions first, three major groups of organisms have been described as important in the rapid oxidation of sulphur in soil (Keunen 1975; Wainwright 1978) and are described as:

1. the colourless sulphur bacteria including three families, Thiobacteriaceae, Beggiatoaceae and Achromatiaceae (see Table 1);
2. photosynthetic S-bacteria (Chromatiaceae and Chlorobacteriaceae); and
3. heterotrophic microorganisms which do not gain energy from the oxidation of sulphur compounds (some actinomycetes, bacteria, and fungi).

The groupings most important (Wainwright 1978) in turnover of sulphur in soil are 1. and 3., above. Although group 2. is generally involved in aquatic systems, Wainwright (1978) clearly pointed out that they could play a role in flooded soils systems and will therefore be included in our discussion.

2.1.1.1 Colourless sulphur bacteria. Very little is known about the physiology of the bacteria listed in Table 1, because obtaining consistent pure cultures for many of them has been difficult (Keunen 1975). Our discussion is thus restricted to those genera which have been isolated and studied in pure culture. These are: (1) Thiobacillus; (2) Thiomicrospira; (3) Sulfolobus; (4) Thermothrix; and, (5) Beggiatoa (Keunen 1975; Brock et al. 1984).

Table 1. The colourless sulphur bacteria.

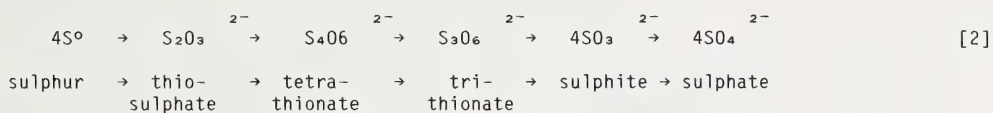
Thiobacteriacea	Beggiatoaceae	Achromatiaceae
Thiobacterium	Beggiatoa	Achromatium
Macromonas	Thiospirillopsis	
Thiovulum	Thioploca	
Thiospira	Thiothrix	
Thiobacillus	Thiodendron	
Thiomicrospira		
Sulfolobus*		
Thermothrix		

*now is considered an archaebacteria
Source: Keunen (1975)

2.1.1.1.1 Genus Thiobacillus. This is by far the best studied genus of the sulphur oxidizing bacteria. By nature, these organisms are chemoautotrophs, i.e., they are able to oxidize reduced or partially reduced sulphur compounds (S^{2-} , $S_2O_3^{2-}$, $S_4O_6^{2-}$, S^0) to SO_4^{2-} in the presence of oxygen and gain energy for growth in the form of ATP and fix CO_2 from the atmosphere for cellular constituents. Although only a few species of the thiobacilli are considered important in sulphur oxidation in soil, workers have classified these microorganisms in different ways. In one scheme, Starkey (1966) grouped and differentiated the bacteria on the basis of their chemical reactions performed (Table 2). Other workers have categorized the thiobacilli on the basis of their nutritional requirements and characteristics such as obligate chemolithotrophy (Keunen and Beudeker 1982).

Perhaps a more simplified and unifying approach, though, is that of grouping the bacteria according to the pH where their biological activity is at a maximum. Here two groupings can be seen; those living at neutral pH and those preferring an acid environment. Laishley et al. (1978) adopted the terms "less acidophilic" and "acidophilic" to describe these groupings. The differential characteristics of the bacteria within these groups are summarized in Tables 3 and 4. As can be seen, it is becoming necessary to study each new organism in as much detail as possible; classification based simply on chemical reactions or on nutritional characteristics is not sufficient to differentiate new isolates.

As mentioned earlier, the thiobacilli are unique in that they can grow lithotrophically (i.e., gain energy from oxidation in inorganic compounds) on reduced inorganic sulphur compounds. Although the pathway(s) involved in the oxidation of these reduced compounds have not been completely agreed upon, the polythionate pathway determined from pure culture studies and soil experiments seems to be the one most accepted (Peck 1960; Nor and Tabatabai 1977; and Wainwright 1978):



It should be pointed out, however, that any one species of thiobacilli may not possess all components of this pathway. For example, it is known in our studies with T. kabobis (Reynolds et al. 1981) that the products from the oxidation of $S_2O_3^{2-}$ are $S_4O_6^{2-}$ and SO_4^{2-} ; the $S_4O_6^{2-}$ formed was not reoxidized to SO_4^{2-} . It is probably better to think of the oxidation reactions in a more flexible manner and consider the various ways in which the inorganic sulphur compounds may be oxidized to SO_4^{2-} (Figure 2). Brock et al. (1984) suggested that the oxidation of sulphide (S^{2-}) and sulphur (S^0) to SO_4^{2-} is brought about by reaction of these compounds with cell bound sulphhydryl groups, with the sulphur subsequently being oxidized to the key intermediate sulphite (SO_3^{2-}). The energy (ATP) for growth and metabolism can then be obtained in one of two ways. The first is via a sulphite oxidase system which is presumed to be present in all thiobacilli. Electrons from the oxidation reaction are funnelled into an electron transport system composed of flavoproteins, quinones, and cytochromes. It has been shown that there are two positions in the chain where there is enough energy released to synthesize the important high

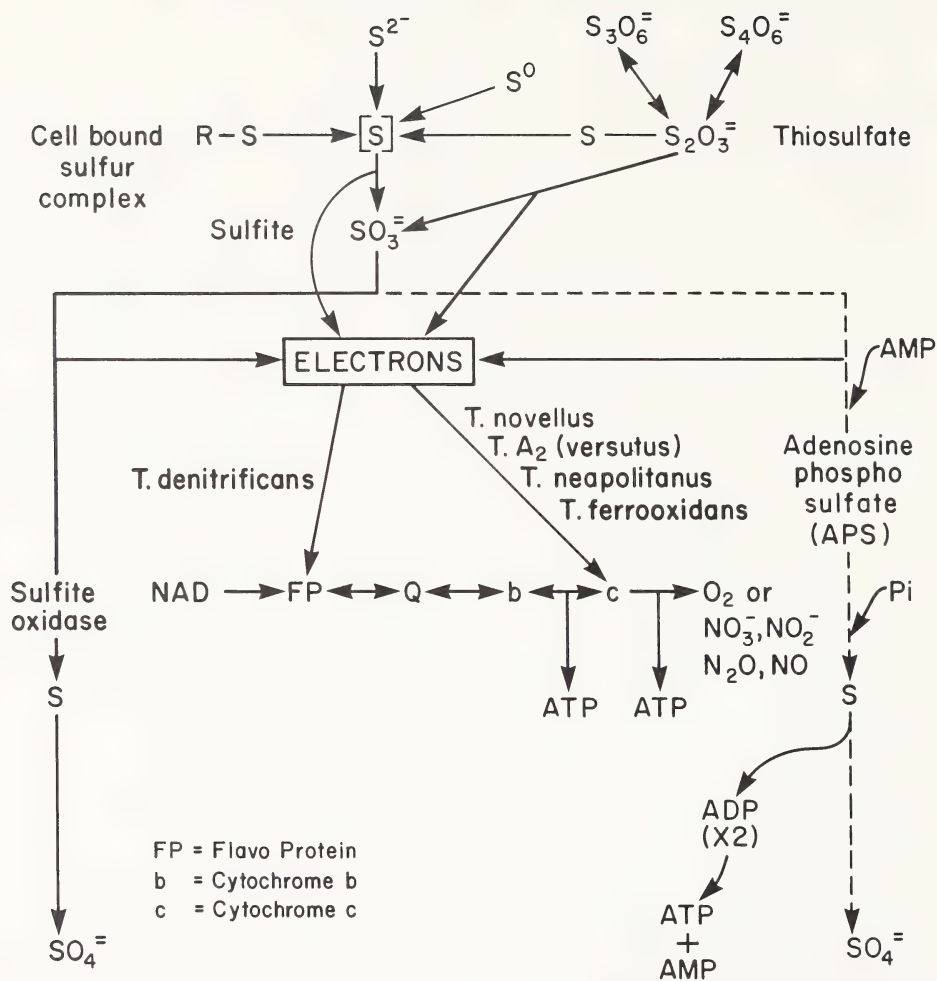


Figure 2. Ways in which inorganic sulphur compounds may be oxidized to SO_4^{2-} (modified after Laishley et al. 1984; Brock et al. 1984; and Kelly 1982).

Table 2. Chemical reactions of the thiobacilli.

(1)	$2S^0 + 3O_2 + 2H_2O$	→	$2H_2SO_4$
(2)	$Na_2S_2O_3 + 2O_2 + H_2O$	→	$Na_2SO_4 + H_2SO_4$
(3)	$2Na_2S_4O_6 + 7O_2 + 6H_2O$	→	$2Na_2SO_4 + 6H_2SO_4$
(4)	$2KSCN + 4O_2 + 4H_2O$	→	$(NH_4)_2SO_4 + K_2SO_4 + 2CO_2$
(5)	$5S + 6KNO_3 + 2H_2O$	→	$3K_2SO_4 + 2H_2SO_4 + 3N_2$
(6)	$5Na_2S_2O_3 + 8NaNO_3 + H_2O$	→	$9Na_2SO_4 + H_2SO_4 + 4N_2$
(7)	$12FeSO_4 + 3O_2 + 6H_2O$	→	$4Fe_2(SO_4) + 4Fe(OH)_3$

<u>Bacterium</u>	<u>Reaction(s) carried out</u>
<u>I. denitrificans</u>	1, 2, 3, 4, 5, 6
<u>I. thioparus</u>	1, 2, 3, 4
<u>I. thiooxidans</u>	1, 2, 3,
<u>I. ferrooxidans</u>	1, 2, 7
<u>I. novellus</u>	2, 3

Source: Starkey (1966).

Table 3. Characteristics of less acidophilic thiobacilli.

	<u>I. capsulatus</u> (New isolate-to be published)	<u>I. versutus</u> ¹	<u>I. denitrificans</u> ²	<u>I. intermedius</u> ³
<u>Ultrastructure</u>				
<u>Cell morphology</u>				
Cell size (μm)	0.3 x 0.5-0.8	0.6-0.7 x 1.0-1.3	0.5 x 1.3	0.6-0.8 x 1.0-1.4
Gram-negative cell wall	+	+	+	+
Flagella	Single polar	Tuft polar	Single polar	Polar
Pili		+	-	N.D.
Glycocalyx	+	N.D.	N.D.	N.D.
<u>Cytoplasmic granules</u>				
Sulphur	Rare	N.D.	+	N.D.
Volutin	+		+	N.D.
Poly- β -hydroxybutyrate	N.D.	N.D.	N.D.	N.D.
Carboxysomes	+	N.D.	N.D.	+
Paracrystalline structures	-		-	+
Lamellar bodies		-		-
<u>Growth substrates</u>				
S ⁰	+	Weak +	N.D.	+
S ₂ O ₃ ²⁻	+	+	+	+
S ₂ O ₆ ²⁻	+	N.D.	N.D.	N.D.
S ₄ O ₆ ²⁻	+	-	+	+
S ₂ O ₃ ²⁻	+	+	+	N.D.
FeSO ₄	-	N.D.	N.D.	N.D.
Carbon compounds		+		-
<u>Physiology</u>				
pH growth range	5.5-7.0	7.5-8.0	N.D.	5.5-6.0
Strict aerobe	+		-	+
Optimum growth temp., °C	26-30	30-37	N.D.	30-35
Autotrophic colony growth on agar media	Non-pigmented rosebud (1-2 mm)	Creamy white opaque (2 mm)	Thin, clear (1-2 mm)	Yellow, opaque (1 mm)
Motile	+	+	+	+
% G + C	54.5	67.0	63.3	64.9

continued...

Table 3 (Continued).

Ultrastructure	<i>I. neopolitanus</i> ⁴	<i>I. novellus</i> ⁵	<i>I. thiocyanoxidans</i> ⁶	<i>I. thioparus</i> ⁷	<i>I. perometabolis</i> ⁹
<u>Cell morphology</u>					
Cell size (μm)	0.50 x 1.0-1.5	0.7-0.8 x 1.0-1.5	0.3-0.5 x 0.5-1.5	0.3-0.7 x 0.4-1.2	0.4-0.5 x 1.1-1.7
Gram-negative cell wall	+	+	+	N.D.	+
Flagella	-	-	Single or paired	Single polar	Single polar
Pili	N.D.	N.D.	N.D.	N.D.	N.D.
Glycocalyx	N.D.	N.D.	N.D.	N.D.	N.D.
<u>Cytoplasmic granules</u>					
Sulphur	N.D.	+	N.D.	N.D.	N.D.
Volutin	+	+	N.D.	+	N.D.
Poly- β -hydroxybutyrate	-	N.D.	N.D.	N.D.	N.D.
Carboxysomes	+	N.D.	N.D.	+	N.D.
Paracrystalline structures	-	-	-	-	N.D.
Lamellar bodies	-	-	-	+	N.D.
<u>Growth substrates</u>					
S ^o	N.D.				
S ₂ O ₃ ²⁻	+	+	+	+	+
S ₂ O ₆ ²⁻	N.D.	N.D.	N.D.	+	N.D.
S ₄ O ₆ ²⁻	N.D.	+	N.D.	+	+
SO ₃ ²⁻	+	+	+	+	N.D.
FeSO ₄	N.D.	N.D.	N.D.	N.D.	N.D.
Carbon compounds	+	+	-	-	+
<u>Physiology</u>					
pH growth range	6.2-7.0	7.0	7.0-7.2	6.6-7.2	5.5-6.0
Strict aerobe	+	+	N.D.	-	+
Optimum growth temp., °C	28	25-30	22	28	35-37
Autotrophic colony growth on agar media	White-yellow (1-2 mm)	Colourless-become white (1 mm)	Canary yellow (1-2 mm)	White-yellow (1-2 mm)	White, opaque (1-3 mm)
Motile	-	-	+	+	+
% G + C	52.3	67.3	63.0	62.0	65.0

continued...

Table 3 (Concluded).

1. I. versutus (ATCC 27793). Also known as I. rapidicrescens. Taylor and Hoare (1969); Korhonen et al. (1978); Katayama-Fujimura et al. (1983a, 1983b).
2. I. denitrificans (ATCC 23642). Shively et al. (1970); Taylor and Hoare (1971); Buchanan and Gibbons (1974); Katayama-Fujimura et al. (1982, 1983b).
3. I. intermedius (ATCC 15466). London (1963); Shively et al. (1970); Shively (1974); Katayama-Fujimura et al. (1983b, 1984).
4. I. neopolitanus (ATCC 23640). Parker and Prisk (1953); Beudeker et al. (1981); Katayama-Fujimura et al. (1983b).
5. I. novellus (ATCC 8093). Santer et al. (1959); Kocur et al. (1968); Taylor and Hoare (1971); Katayama-Fujimura et al. (1983a,b).
6. I. thiocyanoxidans. Happold and Kay (1937); Happold et al. (1954); Katayama-Fujimura et al. (1982).
7. I. thioparus (ATCC 12133). Starkey (1935); Katayama-Fujimura et al. (1983b).
8. I. perometabolis (ATCC 23370). Katayama-Fujimura et al. (1983a, 1983b, 1984).

N.D. = not determined

Table 4. Characteristics of acidophilic thiobacilli.

Ultrastructure	<i>I. albertis</i>	<i>I. kabobisa</i>	<i>I. thiooxidans</i> stains ^b	<i>I. ferrooxidans</i> stains ^c	<i>I. acidophilus</i>
Cell morphology					
Cell size (μm)	0.45 x 1.2-1.5	0.4 x 2.0	0.5 x 1.0-2.0	0.5 x 1.0	0.5-0.7 x 1.1-1.8
Gram-negative cell wall	+	+	-	+	+
Flagella	Tuft polar	Single polar	N.D. ^e	+	N.D.
Pili	-	Single	-	+g	N.D.
Surface subunit layer	+	+	-	-	N.D.
Glycocalyx	+	-	N.D.	N.D.	N.D.
Cytoplasmic granules					
Sulphur	+	+	-	-	-
Volutin (dense bodies)	-	+	+	+	+
Poly- β -hydroxybutyrate	+	-	+	+	+
Carboxysomes	+	+	+	-	-
Glucan	-	-	-	-	+
Growth substrates					
S ^o	+	+	+	+	+
S ₂ O ₃ ²⁻	+	+	+	+	-
S ₄ O ₆ ²⁻	+	-	+	+	N.D.
FeSO ₄	-	-	-	+	-
Carbon compounds	-	-	-	-	+
Physiology					
pH growth range	2.0-4.5(3.5-4.0) ^h	1.8-6.0(3.0)	0.5-6.0(2.0-3.5)	1.4-6.0(2.5-2.8)	1.5-6.0(3.0)
Strict aerobe	+	+	+	+	+
Optimum growth temp., °C	28-30	28	28-30	15-20	25-30
Autotrophic colony growth on agar media	Yellow (1 mm)	Yellow (1-3 mm)	Whitish yellow(1 mm)	Pinpoint	N.D.
Motile	+	+	+	+	+
% G + C	61.5	51.7	52-53	55.0-57.1	62.9-63.2

- a Reynolds et al. (1981)
b Trudinger (1962); Mahoney and Edwards (1966); Shively et al. (1970); Buchanan and Gibbons (1974); Harrison (1982)
c Trudinger (1962); Shively et al. (1970); Buchanan and Gibbons (1974); Shively (1974); Hirt and Vestal (1975); DiSpirito et al. (1982)
d Guay and Silver (1975); Matin and Matin (1982)
e N.D.=not determined
f Depending on what strains are examined - can have single or tufts or polar flagella (DiSpirito et al. (1982)
g Several (DiSpirito et al. 1982)
h Optimum pH parenthesized
Source: Bryant et al. (1983)

energy ATP molecule, both at the level of cytochromes. It seems that the electrons from the aerobic thiobacilli enter the chain at the level of cytochrome C, and at flavoprotein for certain facultative aerobes like I. denitrificans which use NO_3^- as the final electron acceptor when O_2 is absent (Justin and Kelly 1978; Kelly 1982).

A second less common route in which high energy phosphate bonds may be synthesized from the oxidation of SO_3^{2-} is the APS (adenosine phosphosulphate) pathway. It has been found in only a few species and requires that SO_3^{2-} combine first with AMP (adenosine monophosphate) to form APS. The two electrons removed from this reaction are passed to the cytochrome system where ATP is made. A second molecule of ATP is eventually made by a process called substrate level phosphorylation when inorganic phosphate (Pi) reacts with APS to form $\text{ADP} + \text{SO}_4^{2-}$.

A major question relevant to all microorganisms growing chemoautotrophically is how NAD^+ is reduced so that CO_2 may be reduced to cellular material. As yet the only satisfactory explanation comes from the concept of reversed electron transfer in which electrons are pushed upwards against a gradient in the respiratory chain from a positive to negative redox potential at the expense of ATP (Aleem 1969; Gottschalk 1979). This feature explains in part the slow growth generally noted in thiobacilli.

2.1.1.1.2 Genera Sulfolobus, Thiomicrospira, and Thermothrix. The genus Sulfolobus was discovered by Brock et al. (1972) and is generally found in environments possessing the following features:

1. pH 1-5; and
2. temperature 60 - 75°C; usually hot sulphur springs

Besides being located in unusual environments, the genus Sulfolobus possesses other properties not typical of the normal procaryote cell (Woese 1981). First, the lipid components of the cell wall of Sulfolobus are ether-linked whereas the usual procaryote lipid components are ester-linked. Moreover, the cell wall of Sulfolobus lacks peptidoglycan. As this genus also differs from the normal procaryote in its antibiotic sensitivity and translational machinery, it prompted Woese (1981) to propose a new kingdom of life forms called the "Archaeobacteria." Other members added to this kingdom include the methanogenic bacteria and the extremely halophilic bacteria.

Species of the genera Sulfolobus, Thiomicrospira, and Thermothrix can oxidize H_2S to S^0 and finally to H_2SO_4 (sulphuric acid). The ability of Sulfolobus and Thermothrix to withstand a hot environment is generally attributed to an unusual acid and heat stable cytoplasmic membrane composed of long chain hydrocarbons (isoprenoids) (Brock et al. 1984).

The genus Thermothrix is also found around sulphur springs, but unlike Sulfolobus does not prefer strong acid conditions. These species are also capable of anaerobic growth using NO_3^{2-} as an alternate electron acceptor to O_2 .

Not much is known about Sulfolobus or Thermothrix with regard to their biochemistry. However, it is assumed that their mechanism of sulphide oxidation would be similar to the scheme presented for the thiobacilli (Figure 2). Because these organisms are confined to restricted environments they likely do not play major roles in sulphur turnover in forest or agricultural soils.

Species of the genus Thiomicrospira are also not well characterized. One species, Thiomicrospira pelophila, was isolated by Keunen and Veldkamp (1972) and found to be very similar to Thiobacillus thioparus except that it could withstand higher concentrations of H₂S. Ruby et al. (1981) have recently isolated several new strains from vent water rich in sulphide. The involvement of this genus in acidification of soils is unclear but at best would likely be confined to soils which may be flooded.

2.1.1.1.3 Genus Beggiatoa. Like the genera Sulfolobus and Thermotrix, the genus Beggiatoa is confined to niches rich in H₂S. Species have been isolated from sulphur springs, mid layers of lakes, and in water polluted with sewage. These filamentous organisms are probably mixotrophic in that they may use H₂S as an energy source but must rely on organic compounds like acetate or succinate for their carbon rather than on CO₂ (Strohl and Larkin 1978). These bacteria are very dependent on H₂S and oxidize it to elemental S⁰ which often shows up in phase contrast micrographs as round refractile inclusion bodies (Carpenter 1977; Strohl and Larkin 1978). The benefit Beggiatoa obtain from the sulphur deposition and the presumed oxidation of the sulphur granules to sulphate is a topic of great debate (Nelson and Castenholz 1981). Thus far, three main functions have been proposed. First, the oxidation of S⁰ to SO₄ may be a source of energy for cell growth. Secondly, the intracellular S⁰ may serve, under anaerobic growth conditions, as an electron acceptor in reducing the S⁰ back to H₂S (Keunen and Beudeker 1982). Finally, it has been speculated that because Beggiatoa is catalase negative and may produce toxic hydrogen peroxide when growing on organics, the H₂S may act therapeutically by reacting with and removing the toxic intracellular hydrogen peroxide (Burton and Morita 1964; Keunen 1975; Strohl and Larkin 1978; and Nelson and Castenholz 1981).

Again, because Beggiatoa thrives best in still aquatic environments its role in the sulphur cycling in Alberta would be restricted to conditions where flooded soils prevail.

2.1.1.2 Phototrophic sulphur bacteria: anaerobic oxidation of reduced sulphur compounds.

The phototrophic sulphur bacteria are often referred to as the green and purple bacteria and belong to two distinct families named the Chromataceae and Chlorobiaceae, respectively. In contrast to plant photosynthesis, bacterial photosynthesis occurs anoxygenically, uses H₂S (generally) as an electron donor, and produces elemental S⁰, inside or outside the cell. In anaerobic aquatic environments these bacteria oxidize H₂S and S⁰ to SO₄²⁻ and in so doing derive energy (ATP) required for CO₂ fixation (see Figure 1). Because of their requirement for light to mediate the oxidation of H₂S, Stanier (1961) reasoned that the only function of H₂S was to provide electrons for CO₂ assimilation since the ATP was generated by cycling photophosphorylation along an electron transport chain (Figure 3). However, there still remains a great deal of uncertainty as to the way in which NADPH is created for reductive biosynthetic reactions. The most probable mechanisms involve components of the photosynthetic electron transport chain in a non-cyclic way as shown by the dashed lines in Figure 3 (Brock et al. 1984). Nevertheless, overall oxidation of H₂S proceeds in two stages, the first a rapid oxidation inside the cell and the second a slow reaction illustrated as follows (Boyd 1984: 67):

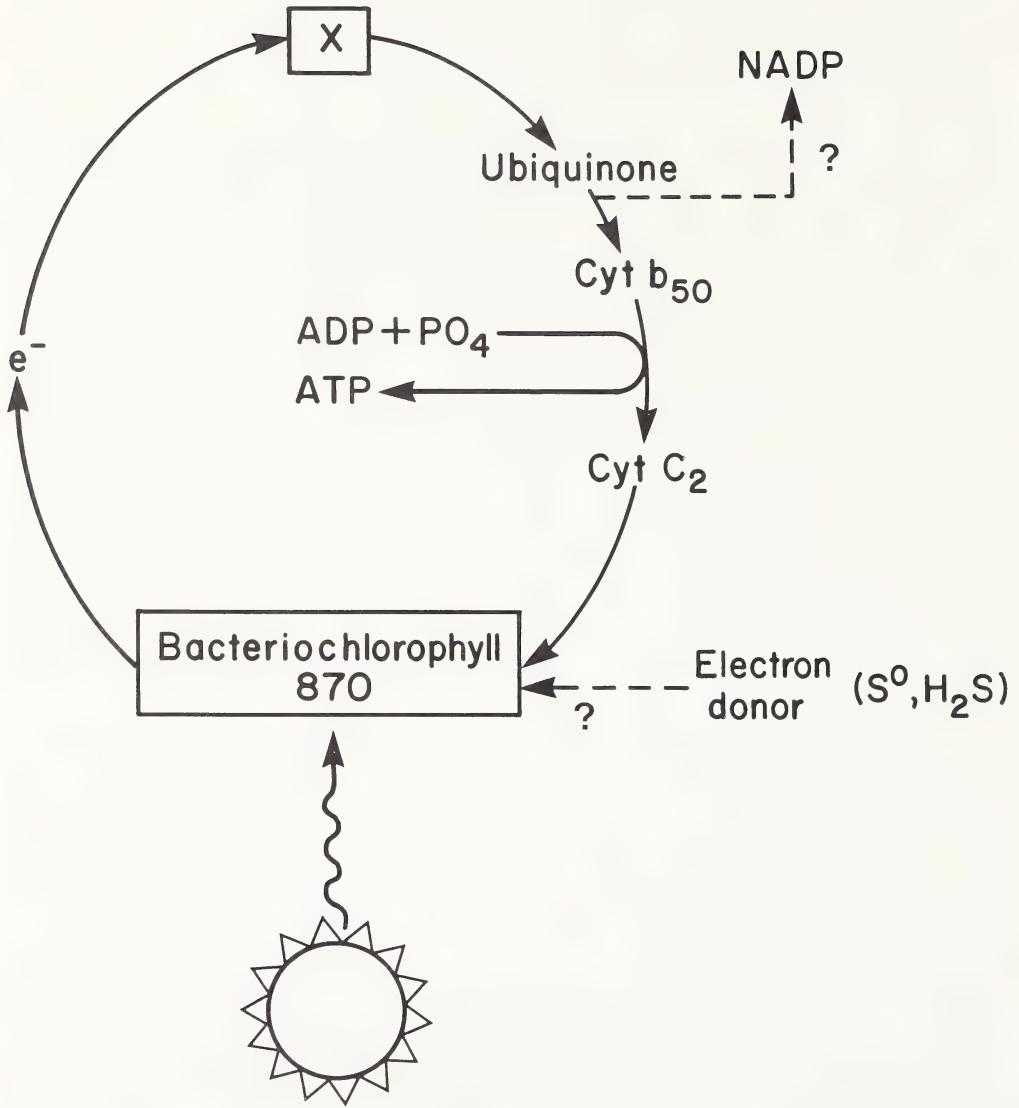
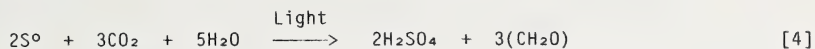
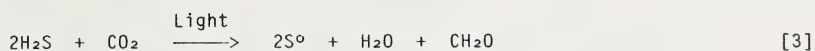


Figure 3. Photosystem in green and purple bacteria (reproduced by permission from Boyd 1984: General Microbiology, St. Louis. Times Mirror/Mosby College Publishing).



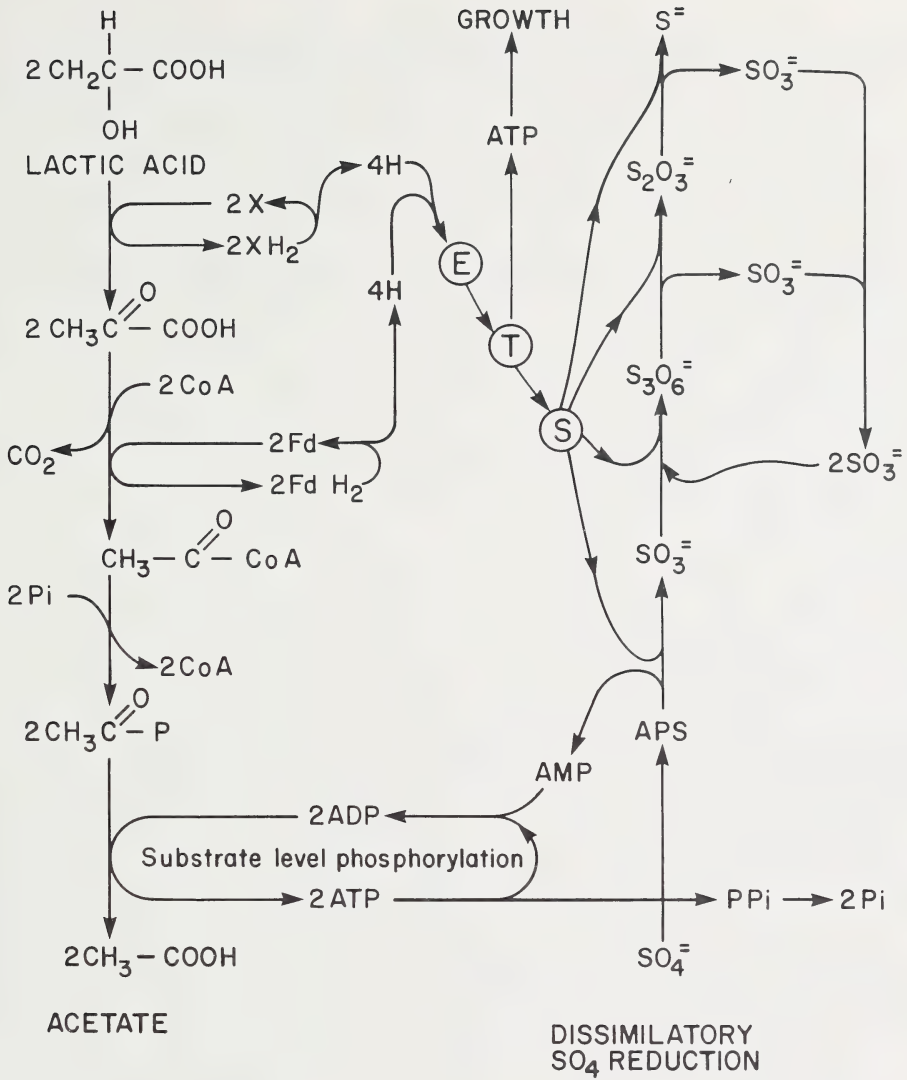
Overall reaction



The rather narrow position or zone of the phototrophic bacteria in aquatic environments is dictated by the simultaneous requirement of light and H_2S or S^0 and minimum O_2 . Baas Becking (1925) used the term "sulfuretum" to describe this rather restricted zone where the bacterial phototrophs grow. Maximum levels of H_2S tolerance place a further restriction on the location of the phototrophs in the sulfuretum. For example, in vitro studies show that Chlorobium has a maximum sulphide tolerance of 4-8 nM while Chromatium has a sulphide tolerance of 0.8-4 nM sulphide (Pfennig 1975). The occurrence of these two generally correlates well with the gradient of sulphide normally observed in stagnant still water with Chlorobium near the bottom where H_2S concentrations are relatively high, and Chromatium near the surface where H_2S concentrations are lower. The H_2S gradient is due to two processes, one reflecting the source where H_2S is produced and the second due to chemical properties of H_2S . Considering the source of H_2S production first, the vast majority is from the reduction of SO_4^{2-} in stagnant waters by the sulphate reducing bacteria (to be discussed later). It is a feature of H_2S and any other compound with sulphydryl groups that they are stable only under anaerobic conditions; as the sulphide approaches the water surface it is quickly auto-oxidized. Essentially then, the H_2S gradient is the inverse of the O_2 gradient.

It is also not too surprising that the phototrophic sulphur bacteria are able to compete very well with the colourless sulphur bacteria for available H_2S (Jorgensen 1982). As with the colourless sulphur bacteria, their roles in the sulphur cycle are generally in aquatic systems but could play a role in any soil which experiences flooding conditions.

2.1.1.3 Heterotrophic microorganisms. Heterotrophic microorganisms including bacteria, fungi, and actinomycetes are capable of oxidizing reduced forms of inorganic sulphur. Wainwright (1978) indicated that bacteria belonging to the genera Arthrobacter, Bacillus, and Flavobacterium could oxidize elemental S^0 or $\text{S}_2\text{O}_3^{2-}$ to SO_4^{2-} in vitro. He further indicated that species like Achromabacter sp. and Pseudomonas sp. could oxidize $\text{S}_2\text{O}_3^{2-}$ to $\text{S}_4\text{O}_6^{2-}$. Reports of fungal and actinomycete oxidation of inorganic sulphur are less common (Wainwright 1979; Germida et al. 1985) but the fungus Penicillium decumbens has been reported to oxidize S^0 to S_2O_3 (Wainwright 1978). At present there is no clear evidence to indicate that the heterotrophic microorganisms play important roles in sulphur cycling. Starkey (1966) states that these reactions are "incidental to their normal metabolism."



Fd = Ferredoxin
ETS = Electron Transport System

Figure 4. Oxidation of lactic acid and the dissimilatory reduction of sulphate by *Desulfovibrio* sp.

1978) and more recently in five other other Clostridial species (Laishley et al. 1984), all of which are non-classical sulphate reducers. Clostridial species have an advantage over the classical dissimilatory reducers in that they contain proteolytic and saccharolytic organisms. This means that they grow on a variety of complex proteins and sugars common in the soil system and they they are not dependent on fermentation products (e.g. lactate) for their metabolism as are the traditional sulphate reducers. The implication here is that the Clostridial species would likely be more ubiquitous than the classical sulphate reducers and that the scope of bacteria carrying out these reactions may be much wider than anticipated. However, these organisms would not play a significant role in agricultural soils unless they became anaerobic due to flooding conditions.

Also in the context of nonclassical sulphate reduction, some species from the genera Salmonella, Proteus, Camphylobacter, Succhuromyces and Pseudomonas have been shown, in pure culture, to anaerobically reduce small amounts of SO_4^{2-} to HS^- (McCready et al. 1974; Brock 1984). However, the role these organisms play in the cycling of inorganic sulphur in the ecosystem has not been determined.

3. ECOLOGICAL AND ECONOMIC ACTIVITIES OF MICROBIAL INORGANIC SULPHUR OXIDATION AND REDUCTION

3.1 OXIDATION REACTIONS

3.1.1 The Colourless Bacteria and Heterotrophs

3.1.1.1 Oxidation of sulphur. Besides the role of producing SO_4^{2-} , the major form of S which plants require, the events of sulphur oxidation reactions have many other effects (some subtle and other dramatic) on the soil system. For example, it is known that as the thiobacilli oxidize elemental sulphur (S^0), sulphuric acid (H_2SO_4) is produced which can cause acidification of soils. As long as the acid production was controlled (i.e., by limiting available sulphur for attack) then a slight increase in soil acidity could be beneficial in that not only more SO_4^{2-} would be available for plant growth, but the acid would also solubilize and make more of the micronutrients (Ca^{2+} , Mg^{2+} , PO_4^{2-} , K^+) available to terrestrial and aquatic life forms (Gorham 1976; Wainwright 1978). A second advantage of the S^0 oxidation process by the thiobacilli might be the improvement of the fertility of basic soils and in one case the control of the disease "potato scab" (Starkey 1966). Improvement of basic soils could be important in Alberta where tracts of soil are basic in nature with a pH between 8 and 9. With this in mind it has been found that addition of elemental sulphur to soil results in a rapid reduction in soil pH (Adamczyk-Winiarska et al. 1975; Bollen 1977; and Legge et al. 1986). It is therefore theoretically possible to control the oxidation by limiting the amount of applied sulphur to these soils so that the naturally occurring sulphur oxidizers (in particular the thiobacilli) can produce just enough H_2SO_4 to reduce the soil pH to approximately 7. This controlled process could even be accelerated by inoculating the sulphur with a Thiobacillus sp. prior to its application to the soil, a technique which could be thought of as "an agricultural contact C" (Hyne 1981).

Of course, if too much sulphur is applied to the soil then conditions are ideal for producing excess acid via the oxidation reactions of the thiobacilli. Such situations are not uncommon in Alberta where huge stockpiles of sulphur and numerous sour gas processing plants exist which produce great quantities of loose powdered sulphur dust. The sulphur dust can be swept up in prevailing winds and deposited on soils downwind from the sulphur source and be subject to biological oxidation. Such a phenomenon is well documented in the Alberta Whitecourt study where the most severe acidification occurred in the soil profile closest to the gas plant (Legge et al. 1986) and which contained large populations of thiobacilli. In contrast, the indigenous thiobacilli population was barely detectable at the reference (control) soil site.

This soil sulphur acidification phenomenon is brought about by a succession of different Thiobacillus species. The species present depend on their ability to withstand changing physiological growth parameters, because no one species can grow over the wide pH range (i.e., 10 - 0.5) attributed to the thiobacilli. This succession of Thiobacillus species was elegantly shown by Parker (1947) to occur in degradation of concrete sewer pipes. The pipes were first attacked by less acidophilic thiobacilli which oxidized the reduced S compounds, producing an acidic environment conducive for growth of the acid loving (acidophilic) thiobacilli. The acidophilic thiobacilli then further oxidized the

S compounds, producing extremely acid soil conditions in the range of pH 2.5 to 3.0 (Laishley and Bryant 1985).

Copious inputs of acid into the soil system, be it from acidic deposition or biological oxidation of sulphur or other sources, can lead to or induce a number of other biotic and abiotic events in the soil system. There can be little doubt that a lowered environmental pH can adversely affect the soil microflora. One effect of such acidification has been to reduce the heterotrophic bacterial populations (Adamczyk-Winiarska et al. 1975; Wood 1975; Bryant et al. 1979; and Wainwright 1979). Bryant et al. (1979) showed that the respiratory activities of the flora responsible for degrading substrates like glucose, starch, cellulose, casein, or urea were significantly reduced in soils exposed to acid (pH 3.0). It was suggested that these effects of acid may be a common phenomenon, as a local garden soil which was artificially acidified in the laboratory showed similar respiratory responses in degrading these substrates. A second, very important point emerged from this paper. When looking at respiration of acidified and control garden soils, no differences were detected until the potential of soil microbial populations to degrade common substrates like cellulose, casein, and urea were examined. This demonstrated the critical importance of examining more than one parameter when assessing the effect of a potential pollutant on soil activity. In other studies, workers like Tamm (1976) reported that acidification inhibited the nitrification process in a forest soil while others have demonstrated that nitrogen fixation was inhibited under acidic conditions (Oden 1971; Dochinger and Seliga 1975). In addition, Tamm (1976) suggested that the mycorrhizal fungi are very sensitive to acid conditions.

Acid soil may have certain effects on the abiotic components of the soil, in particular the nutrient status. Calcium is positively correlated with productivity in soils and especially forest soils (Wainwright 1980). Indeed, Ca^{2+} losses have been noted as a result of leaching due to H^+ ions which eventually exhaust the soil's cation exchange capacity (Overrein 1972; Abrahamsen et al. 1975). As a note of explanation here, soils contain negatively charged colloids which have attached to them cations including Ca^{2+} , K^+ , and Mg^{2+} . These immobilized cations are then made available to plants for growth by a process called cation exchange, but, when soils become acid the absorbed cations are replaced with H^+ ions and are subsequently leached from the soil system. Ensuing plant death often results (Reuss 1975) and those soils with the weakest cation exchange capacities would likely be most sensitive (Malmer 1976). In contrast to this, soils which are alkaline in nature would have high buffering capacity and would likely be more resistant to the effects of acid (Laishley 1985). Thus two factors affecting a soil's sensitivity to acid would be its cation exchange capacity and its state of alkalinity.

Wherever acid soils are encountered, iron and aluminum mobilization and resulting toxicity to plants is often reported (Rorison 1973; Metson et al. 1977; Francis 1982; and Reuss 1983). These toxicities are due to the actual breakdown of the clay and organic colloid material, which contains large quantities of aluminum and iron, and the subsequent precipitation of soil and plant phosphate.

Finally, in regard to acidification, the most obvious effect would be a direct one in which the H^+ ions would cause direct injury to plant tissues and perhaps impair their ability to absorb important ions like Ca^{2+} , Mg^{2+} , and PO_4^{2-} (Wainwright 1978). Here

one can observe a cascade type of effect; the diminished ability of plants to take up Ca^{2+} may be magnified by the loss of available calcium through leaching processes.

In closing, one should point out that nutrient losses are harder to demonstrate in agricultural soils which are continually under heavy regimens of fertilizer treatment. This does not mean, however, that agricultural soils are immune to the effects of acidification, but rather that the potential dangers are artificially masked by continued nutrient inputs by man.

3.1.1.2 Oxidation of metal sulphides in soil. A number of environmental and agricultural problems occur as the result of chemolithotrophic oxidation of pyrite (FeS_2), a mineral sulphide compound commonly present in coal and coal mine effluent. Pyrite is also found in soils where H_2S from bacterial sulphate reduction and ferrous iron react to form ferrous sulphide (FeS) which subsequently may react with either elemental sulphur (S^0) or sulphides (S^{2-} or HS^-) to form pyrite (Metson et al. 1977). Pyritic soils represent potential sites for acid soil referred to as "mud clay" and may convert to an actual acid soil (called "cat clay") if the environmental conditions change from anaerobic to aerobic (Metson et al. 1977). Under aerobic conditions pyrite becomes oxidized to sulphuric acid, jarosite [$\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$], ferric oxides, and ferric sulphate [$\text{Fe}_2(\text{SO}_4)_3$] (Bloomfield and Coulter 1973; Metson et al. 1977; and Kargi 1982). The jarosite, ultimately formed by chemical reactions with pyrite, oxygen, K^+ , and ferric hydroxide, gives the acid soil a characteristic yellow colour called "yellow mud" or "yellow boy" (Metson et al. 1977). Pyrite may also be subject to oxidation by certain species of thiobacilli. Here two basic schemes are involved and are referred to as direct and indirect oxidation mechanisms (Silverman 1967). In the direct mechanism, I. ferrooxidans oxidizes the pyritic sulphur to sulphate and the ferrous (Fe^{2+}) iron to the ferric (Fe^{3+}) state. In so doing, I. ferrooxidans obtains the energy it requires for growth. The insoluble pyrite is thus transformed to the soluble ferric sulphate [$\text{Fe}_2(\text{SO}_4)_3$] and sulphuric acid as shown in Figure 5 (reaction 1).

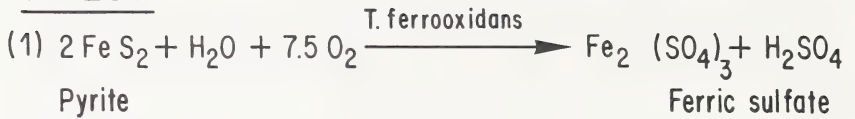
In the indirect mechanism the bacteria oxidize the ferrous (Fe^{2+}) iron to the ferric form (Fe^{3+}), which, being a strong oxidizing agent, solubilizes more of the pyrite to ferrous sulphate (FeSO_4) and sulphur (S^0) (Figure 5, reactions 2 and 3). The end products of these reactions are subject to further reactions by I. ferrooxidans. In reaction 4, the S^0 is oxidized by the usual means to sulphuric acid (H_2SO_4), and in reaction 5, the sulphuric acid from reactions 2 and 4 may react with the ferrous sulphate from reaction 3 and form more of the strong oxidant, ferric sulphate. Of course, in the absence of I. ferrooxidans, only the indirect mechanism of pyrite oxidation is operational and in this case the rate of formation of ferric sulphate would be the rate determining step; without I. ferrooxidans this reaction would be considerably slower.

Other acidophilic thiobacilli (Table 4) may also participate in the oxidation of the elemental sulphur produced in reaction 3 (Figure 5) and thereby catalyze the rapid formation of acid (H_2SO_4), which, in addition to optimizing the catalytic properties of ferric sulphate by keeping Fe^{3+} soluble, can also cause corrosion problems of any metal, stone, and concrete in the vicinity (Parker 1947; Bryant et al. 1985).

Such bacterial oxidation processes, while detrimental in nature, can be used to man's advantage. For example, pyrite in coal can cause problems when coal is combusted

DIRECT AND INDIRECT OXIDATION MECHANISMS FOR PYRITE OXIDATION

DIRECT



INDIRECT

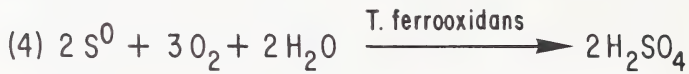
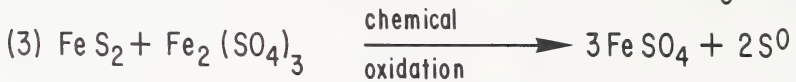


Figure 5. Direct and Indirect Oxidation Mechanisms for Pyrite Oxidation.

and emits sulphur gases. Prior to combustion, techniques are now being employed to remove pyrite from the coal by a process called microbiological desulphization (Kargi 1982).

In another beneficial role, *I. ferrooxidans* is involved in the leaching of copper from low grade ores (Brierley 1978). Basically, the bacteria gain energy for growth by oxidizing ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). In an acid solution the ferric iron functions as a powerful oxidizing agent which solubilizes the metals, making more ferrous iron available for microbial attack. Thus, the bacteria catalyse the leaching process by continually producing the ferric (Fe^{3+}) form of iron.

Thus far, it has been demonstrated that the oxidation of sulphur and metal sulphides can have profound detrimental environmental outcomes. However, the benefits of study and understanding the processes involved in these detrimental events show up in certain industrial and agricultural applications.

3.1.2 The Phototrophic Sulphur Bacteria

By oxidation of H_2S to SO_4^{2-} in anaerobic environments the photosynthetic sulphur bacteria provide the sulphate reducing bacteria with their necessary substrate for growth and do so without the consumption of molecular oxygen (Pfennig 1975; Postgate 1982). As well, these CO_2 -fixing bacteria perform a role in secondary primary production as food sources for the protozoa in lakes (Sorokin 1970; Pfennig 1975).

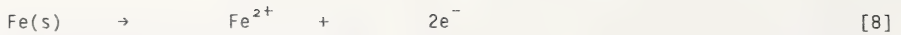
Postgate (1982) even suggested that, under certain water polluted situations, the sulphate reducing bacteria may produce so much H_2S that the development of large colourful phototrophic blooms may occur; these bacteria may therefore function as indicators of pollution. Furthermore, in such situations the phototrophs may also produce high levels of H_2SO_4 and thereby participate in lowering the environmental pH; the effects of such acid production have been discussed at length above.

In terms of their contribution to ecology and economics the phototrophic sulphur bacteria play lesser roles than the colourless sulphur bacteria or the sulphate reducers. However, Nugent (1984) in talking about photosynthetic reactions (which included in addition to the photosynthetic bacteria, the plants, algae, and cyanobacteria), referred to these organisms as "the basic elements of the earth's ecology ultimately providing a great proportion of the food, biomass, and oxygen from simple precursors and light."

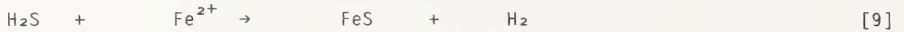
3.2 REDUCTION REACTIONS

The sulphate reducing bacteria complete the sulphur cycle by taking the product of sulphur oxidation (i.e., SO_4^{2-}) and converting it to H_2S by a process called dissimilatory sulphate reduction. The hydrogen sulphide is subsequently used by the sulphur oxidizing bacteria and ultimately recycled as S^0 and SO_4^{2-} . These reactions are possible in a neutral pH environment and stop under acid conditions because the sulphate reducing bacteria are acid sensitive.

However, the activities of the sulphate bacteria are directly linked to the corrosion of iron which costs industry billions of dollars annually in the replacement of corroded metal products. Basically, there are two electrochemical mechanisms by which the sulphate reducing bacteria accelerate the corrosion of iron. In considering the first mechanism an equilibrium oxidation reaction occurs between the iron in the metal and that iron which solubilizes when a metal is immersed in water (reaction 8).



The biologically produced hydrogen sulphide may then interact with Fe^{2+} and produce the typical blackened metal film deposit of FeS (reaction 9)



which somehow maintains the electrochemical process (King and Miller 1971).

In a second less well understood mechanism, it is suggested that the sulphate reducing bacteria may obtain electrons for the reduction of SO_4^{2-} from oxidation of the metal cathodic hydrogen. This mechanism, which seems to appear in most of the literature (King and Miller 1971; Postgate 1979; and Hamilton 1983), seems confusing. First, terms like anodic reductions, anode, and anions are used extensively and although similar in sound they have different meanings. It would be much simpler to refer to the reactions simply as oxidations or reductions. Secondly, terms like polarization and depolarization are never clearly explained. It is particularly with this last term that questions are raised. It suggested that the Desulfovibrio depolarize the cathode by removing the built-up hydrogen film on the metal, thereby obtaining the necessary electrons for the reduction of SO_4^{2-} to H_2S . However, it should be remembered from the physiology of SO_4^{2-} reduction that SO_4^{2-} must first be activated by ATP to form APS (Figure 4). Without this activation SO_4^{2-} does not get reduced. The essential ATP is derived from the oxidation of lactate (or other carbon source) to acetate and electrons from the oxidation function in the reduction of APS. In short, because the reduction of SO_4^{2-} to H_2S is impossible without a carbon source, which in its oxidation provides ATP for SO_4^{2-} activation and electrons for APS reduction, it makes little or no sense that the microbes would waste extra energy in looking for another source of electrons (i.e., polarized hydrogen film on metal).

There is little doubt that the exact method of corrosion of iron is not clear (Hamilton 1983), although Postgate (1982:590) compromised with the statement that "the consumption of cathodic hydrogen by Desulfovibrio and the generation of sulphide contributed to the process".

Apparently, the sulphate reducing bacteria are also prominently involved in the pollution of water. This is particularly obvious around large population centres where large inputs of waste material into local waters provide the necessary carbon and sulphate required for reductive processes to occur on a large scale (Jorgensen 1982; Postgate 1982). Large amounts of H_2S are produced which contribute to the development of the phototrophic sulphur bacteria, and increase the biological oxygen demand.

It can, therefore, be seen in our discussion of oxidation/reduction reactions that shifts or emphasis on certain components of the sulphur cycle can have dramatic ecological and economic repercussions. These situations can be particularly serious, for once the sulphur cycle is imbalanced it is very difficult to intercede and reverse or stop the processes without an input of large numbers of dollars in prevention and reclamation work and, in some cases, a change in man's way of managing his environment.

4. FACTORS AFFECTING THE MICROBIAL OXIDATION OF SULPHUR

It has been shown by Legge et al. (1986) that large amounts of elemental sulphur may be deposited several kilometres downwind from source points (e.g., loose powdered sulphur on existing stock piles or loose powdered sulphur from prilling operations) here in Alberta. This sulphur of course represents a substrate for growth of sulphur oxidizing bacteria. Because these bacteria produce sulphuric acid as a byproduct, the potential for the creation of acid sulphate soils is real. Obviously, the importance of understanding the factors influencing the oxidation of elemental sulphur in soil cannot be understated, as recommendations for the location of future processing plants and sulphur stockpiles or regimens for control and treatment of biologically acidified soils may be possible outcomes.

There are three main features which influence the oxidation of elemental sulphur and these are: (1) the sulphur itself; (2) the sulphur oxidizing microorganisms; and, (3) the nature of the soil environment where sulphur oxidation is occurring (Weir 1975). Each of these areas will be considered in more detail.

4.1. SULPHUR

4.1.1 Composition

Sulphur is a very complex and non-homogenous element. The most abundant and stable class of S^0 under normal ambient conditions is S_8^α , consisting of a crown-shaped, eight membered sulphur atom ring arranged in an orthorhombic crystal lattice (Meyer 1976). Small amounts of polymeric sulphur S_μ (helical chains up to 300,000 sulphur atoms long) (Tobolsky and MacKnight 1965) and S_x composed of a mixture of non- S_8 membered rings (S_6 to S_{26}) and non-crystalline amorphous S_8 (Steudal 1982) make up the other two major molecular classes found in S^0 . The percentage composition of these molecular classes found in S^0 are dependent on the thermal history and mode of solidification (Hyne and Kobryn 1981).

In experiments to look at the effect of molecular composition on bacterial sulphur oxidation, Laishley et al. (1984) first purified production grade sulphur (99.95%) further by the method of Bacon and Fanelli (1942) to the point where it contained 1 ppm impurities. This sulphur referred to as "B and F" had a molecular composition of 91.4% S_8^α , 8.3% S_x and 0.3% S_μ . A second class of sulphur was prepared by subjecting molten B and F S^0 (180°C for 20 min) to a rapid cooling (quench) in liquid nitrogen which had the effect of increasing the percentage of S_x and S_μ . This sulphur was called MMS (mixed molecular sulphur) and had a molecular composition of 71.6% S_8^α , 17.9% S_x and 10.5% S_μ . A third class of sulphur was obtained by extracting S_μ from MMS with CS_2 . This sulphur was called polymeric sulphur (S_μ) and its composition was 100% S_μ .

Laishley et al. (1984) showed that the B and F sulphur and the S_μ (polymeric sulphur) were oxidized by Thiobacillus albertis at similar rates while the MMS sulphur was oxidized at a much slower rate. It was clearly shown that the rate curves for these sulphur forms began to diverge only after some 5% of the total sulphur was consumed (3 days). However, MMS contained S_8^α and S_μ species well in excess of this percentage, indicating that the effect of the different molecular species in MMS was not simply

related to the amount present. Rather, the Sx species in the MMS was likely affecting the rate at which the bacteria could oxidize sulphur forms (S_{μ} and S_8^{α}) that are otherwise readily metabolized to SO_4^{2-} . It was suggested that the increase in Sx content could conceivably alter the way in which the sulphur crystal lattice was packaged, thereby reducing the number of sterically favourable binding sites of *T. albertis* growth as compared to the "B and F" control sulphur, resulting in the lower oxidation rate after the initial three days. This effect of molecular composition of sulphur on microbial oxidation rate is not just an academic one, for the composition would vary depending on what time of the year the sulphur was made at the gas plants. For example, the cool temperatures of January might have the same effect of quick cooling the molten sulphur and thereby increase the percentages of Sx and S_{μ} in the sulphur.

4.1.2 Particle Size and Weight

It has been known that particle size of the sulphur influences the microbial oxidation rate (i.e., the smaller the particle size the faster the oxidation rate). Laishley et al. (1983) have further noted that particle size also determines the total amount of S which can be converted to sulphuric acid. Specifically, *T. albertis* metabolized approximately 70% of the added powdered sulphur (particle size 150 μm - 250 μm) as compared to 3% of an equivalent weight of prilled sulphur (particle size 1.68 mm-2.00 mm). Such information and critical studies with prilled sulphur of varying surface areas was instrumental in establishing the relationship that the microbial oxidation rate of sulphur was a function of surface area per unit weight of sulphur (Laishley et al. 1-983).

This principle of sulphur oxidation correlating with surface area per unit weight of sulphur has a practical application; bacterial sulphur oxidation is not going to occur in an important way unless the available sulphur surface area to unit weight ratio is large, as is the case with wind-blown powder sulphur from huge sulphur storage blocks. This also explains why the towering sulphur blocks, where the surface area/unit weight sulphur ratio is small, have not produced "oceans" of biologically produced sulphuric acid over the last 25 years.

A recommendation for reducing elemental sulphur environmental contamination via microbial oxidation is to continue to store the sulphur in the solid block form and minimize the loose powdered sulphur which is created, for example, as a result of sulphur remelt operations, prilling operations, or transport of sulphur.

4.2 SULPHUR OXIDIZING MICROORGANISMS

As soils differ in the number and types of sulphur oxidizing microorganisms, so too do they vary in their natural abilities of oxidizing elemental sulphur (Moser and Olson 1953; Weir 1975). In addition to this, local environmental pH conditions dictate which sulphur oxidizers will be dominant. For example, the acidophilic thiobacilli would not be actively involved in soils with basic pH and conversely, it would be unusual to detect less acidophilic thiobacilli playing dominant roles in extremely acid conditions. Even if soil conditions are ideal to support the thiobacilli, it should not be forgotten that the acidification process generally takes place through a succession of thiobacilli (Parker 1947; Laishley and Bryant 1985), beginning with the less acidophilic species and

ending with the more acid tolerant species. These events of course do not happen overnight and are strongly dependent on the soil environment (see below).

An interesting area of study has centred on finding how the thiobacilli attach to the hydrophobic sulphur. Takakawa et al. (1979) showed that the initial attachment of Thiobacillus thiooxidans to S^0 was pH dependent, energy dependent, and involved a chemical interaction between the sulfhydryl groups on the cell envelope and elemental sulphur. However, in studies with Thiobacillus albertis, Bryant et al. (1984) found that the attachment to sulphur was not dependent on physiological conditions such as pH, cellular energy, or peripheral cell envelope thio groups but, rather was dependent on the cell's glycocalyx (Costerton and Irvin 1981; Bryant et al. 1983). This threadlike material which radiates outward from the cell wall is probably an acidic mucopolysaccharide polymer (Holt and Beveridge 1982) and has been detected only recently in the thiobacilli via transmission electron microscopy using the antiserum stabilization technique (Bryant et al. 1983) or scanning electron microscopy (Ladd 1982). With regard to Ladd's study (1982), he showed that unidentified thiobacilli attached to rocks by means of glycocalyx material. These thiobacilli were located downstream from a coal mine's effluent creek. In addition, Laishley (unpublished data) has isolated another new, less acidophilic Thiobacillus species which possesses an extensive glycocalyx network, again only microscopically visualized by antiserum stabilization techniques.

We know that under ideal conditions and within the first two days the majority of the thiobacilli cells from the initial culture inoculum are going to be attached at certain sites on sulphur (prilled) by their glycocalyx (Bryant et al. 1983). It was reasoned that microcolony development may occur at these sites whereby the initial adhering cell would divide into two daughter cells after obtaining enough energy from the oxidation of S^0 for fixing CO_2 into cell constituents. This process, repeated many times, would allow biofilms to grow on the surface. Because SO_4^{2-} production occurred at a linear rate over the course of the experiment, it was postulated that only a fixed number of adhering cells are able to metabolize the sulphur, perhaps because of the physical constraints of the S_8^{α} crystal lattice in the solid sulphur or because some cells are impeded from coming in contact with the sulphur by a thick layer of glycocalyx (Laishley et al. 1983). This hypothesis was supported by three pieces of evidence. First, SO_4^{2-} production occurred in a linear fashion. Secondly, approximately 3% of the sulphur prill was oxidized at the end of the experiment and thirdly, scanning electron microscopy showed only cell surface growth of T. albertis (Laishley et al. 1983). For these reasons it is therefore possible that the organism's growth on the sulphur may be somewhat self limiting.

4.3. SOIL ENVIRONMENT

4.3.1 Temperature

Microbial oxidation of sulphur is markedly influenced by temperature. It is generally agreed that oxidation can occur below $10^{\circ}C$, although the rate is very slow (Weir 1975). Maximum rates of oxidation have been reported at $40^{\circ}C$ (Li and Caldwell 1966). However, in contrast to this, a recent work by Bryant et al. (1985) showed that the newly characterized acidophilic Thiobacillus albertis (Bryant et al. 1983) could not

oxidize sulphur at 5°C or 37°C and that its maximum rate of oxidation occurred at 28°C. An interesting feature here was that the organism was killed at 37°C but not at 5°C. In fact, the organism can even survive long periods of freezing (-20°C) and resume its activities when incubated at 28°C.

This is important information for it suggests that to minimize sulphur oxidation, the sulphur should be kept in cool locations. Here in Alberta we are at an advantage with a particularly short growing season and a prevailing cool annual climate. An important point is that if all the sulphur oxidizing microorganisms survive freezing conditions as *T. albertis* does, then each year when conditions warm up the sulphur oxidizing activity would resume, and although the period of sulphur oxidation is short, its effects could be cumulative over the years.

4.3.2 Soil Type

Soil type can have a profound effect on the rate at which S is oxidized. In this regard, buffering capacity is very important, as it tends to dictate how fast the succession of thiobacilli will occur. To examine this feature, sulphur concrete, portland cement, and elemental sulphur cylinders were buried at different test sites throughout the province of Alberta in the fall of 1974 (Laishley 1978). These soil test sites were at Lethbridge, The University of Calgary, Kananaskis Research Station, and Battle Lake and are described respectively, as follows (Laishley and Bryant 1985):

1. Canada Agriculture Research Station, Lethbridge. Classified as a brown agricultural soil.
2. University of Calgary tree nursery. Top soil at this site was removed for many years and now consisted of a subsoil of light dry sand loam (pH 8.5).
3. Kananaskis. Alkaline forest soil (pH 8.2) near Barrier Lake.
4. A slightly acid forest soil (pH 6.75) near Battle Lake, approximately 30 miles southwest of Edmonton.

The data showed that cylinders made of elemental sulphur, or a composite sulphur concrete mix, acted as a substrate for growth and proliferation of the initially non-detectable indigenous thiobacilli population within a short period of time, irrespective of soil type. After several years the thiobacilli were detected around the different sulphur containing specimens, demonstrating the succession of the different thiobacilli types in which the less acidophilic species appear first, preparing an acid environment conducive to the growth of the acid loving acidophiles (Bryant et al. 1983). Even though significant thiobacilli populations were detected in soils surrounding the different sulphur cylinders within the first two years and persisted throughout the test period (8 years up to 1982) at the various field sites, soil acidification occurred at different time periods depending on a number of conditions. At the Lethbridge and Battle Lake sites, drastic soil acidification occurred only around the different sulphur cylinders which were either broken into small pieces by vandals (Battle Lake) or observed to be deteriorating by natural causes (Lethbridge) as compared to a very slow trend in acidification of soils surrounding the intact sulphur containing cylinders at the other sites (see explanation on surface area per unit weight of sulphur above). This microbial

acidification process was in progress for five years at the Battle Lake site before it became evident by measuring pH alone. The more highly buffered Lethbridge soil showed this pattern of development after seven years. It is recognized that the relatively quick acidification of the Battle Lake site may be partly attributed to the fact that forest soils in general are poorly buffered and acidify much more quickly than highly organic agricultural soils (Nyborg 1982). Although the Kananaskis soil had high thiobacilli counts it showed no trend in acidification after eight years due to its high percentage of carbonates.

This finding, that it takes a long time to detect soil pH changes in regions which may be contaminated with sulphur, was also illustrated by Lore (1984). It was found that no pH change occurred in soil profiles over a nine year period near the Waterton Sour Gas Plant in southern Alberta. This, however, does not preclude the existence of large populations of thiobacilli in this soil because they were not measured in this study. As Laishley and Bryant (1985) indicate, the measurement of pH alone tends to give a false sense of security. This was especially true in those studies which showed no significant changes in pH over long periods of time even though active microbial oxidation of sulphur to sulphuric acid was occurring (Laishley and Bryant 1986). In fact, the soil pH determinations only showed soil acidification after the event had happened and the time for this to occur depended on when the soil finally lost its own natural buffering capacity. There is no question that the microbial assay system for thiobacilli (developed by Laishley and Bryant 1985) is a sensitive and rapid test procedure for detecting fugitive sulphur contamination from whatever source and would allow lead time for corrective measures to be employed to prevent potential environmental acidification problems.

4.3.3 Moisture and Nutrients

Sulphur oxidizing microorganisms are strongly affected by moisture conditions. Often in soil, moisture status is closely linked with oxygen status and it is known that the thiobacilli will not grow in water-logged soils. In lab studies it is possible to cut the rate of oxidation of thiosulphate (an alternate H_2O soluble inorganic sulphur compound which the thiobacilli will oxidize) in half merely by doubling the amount of nutrient broth in the culture flasks, all other variables kept constant (Laishley, unpublished data). This demonstrates the critical need for O_2 availability in the oxidation process.

At the Lethbridge Research Station, Laishley and Bryant (1985) observed an interesting phenomenon related to moisture levels - a demonstration of a complete sulphur cycle in operation. One of the buried sulphur concrete cylinders (see Section 4.3.2), which had already developed large populations of thiobacilli in soil adjacent to it, was subjected to spring flooding conditions one season. Consequently the aerobic thiobacilli discontinued growth but the accumulated sulphate product was metabolized by the anaerobic sulphate reducers. Indeed, when the originally pale yellow sulphur concrete cylinder was removed, it was covered with a black iron sulphide (FeS) deposit indicating that the sulphate had been reduced to hydrogen sulphide (Laishley 1978). The original presence and increase in the numbers of thiobacilli indicated that the oxidation reactions were taking place, while the hydrogen sulphide production demonstrated the occurrence of reduction reactions (See Figure 1, Section 2).

Moser and Olson (1953) have also shown that moisture is important in S^0 oxidation and found that moisture levels near field capacity generated maximum microbial oxidation. In agreement with this, Laishley and Bryant (1985) observed at a sandy loam site under very dry conditions (moisture content <2%) at The University of Calgary, that the sulphur oxidizing activity of the thiobacilli in soils around these buried sulphur cylinders was low and the occurrence of these organisms was sporadic over the 8-year study period.

Along with moisture content the nutrient status affects the activity of most plants and soil microorganisms. It is agreed that microorganisms generally need the same nutrients which are essential to plant growth (Weir 1975). In fact, sulphur oxidation may be enhanced by N-P fertilizers (Bloomfield 1967) which presumably augment both plant and sulphur oxidizing bacteria metabolism. It is obvious that soil biological processes are intricate and interrelated and that availability of moisture and nutrients is important in maintaining and perpetuating this activity.

We have therefore seen in this chapter that the factors controlling the oxidation of elemental sulphur are many and varied and when one looks at potential impacts of oxidation of sulphur on soils these variables must be taken into account.

5. THE ALBERTA PROBLEM

Although most of the agricultural soils in the prairie provinces are neutral or slightly basic, this feature does not necessarily hold true in Alberta where large percentages of land are already slightly acidic, in the range of pH 5 to 6 (Penney et al. 1977). In their study, Penney et al. (1977) determined the pH of 88,000 farm soil samples and basically established a map for Alberta showing the percentages of cultivated land with a pH of 6 or less (Figure 6). The alarming point was made that 17% of the cultivated land is already acidic (less than pH 6) and that another 25% could become acidic very soon with a drop in pH of 0.5 to 1.5 units in the top 15 cm. These problems are most serious in the belt running from Calgary to Lloydminster and in the Peace River region (Figure 6).

The seriousness of the pH problem is realized when the productivity of crops is examined. For example, the productivity of some crops like alfalfa and barley were significantly reduced when slight decreases in soil pH were noted (Penney et al. 1977). The productivity of crops from several soil pH ranges were compared to the productivity of soils which had been limed with Ca(OH)_2 such that the final pH was 6.7 in all cases. Some of these data are presented in Table 5 and clearly show the effects of acid on crops; the yields of barley and alfalfa were increased by 98% and 301%, respectively, by liming pH 5 soils to 6.7. From another perspective, it can also be seen that unlimed soils in the pH ranges ≤ 5.0 and 5.1 to 5.5 were far less productive than soils in the pH 5.5 to 6.0 range. After viewing such data one might be left with the impression that all agricultural crops are adversely affected by acid. However, such is not the case - rapeseed and red clover are more acid tolerant and do not show the drastic reduction in crop yield at pH ≤ 5 as do barley and alfalfa (Penney et al. 1977).

In Alberta, the sour gas processing industry removes the contaminating hydrogen sulphide from the gas and converts it to elemental sulphur via the Claus process (Hyne 1977), while the unreacted H_2S is burned and released to the atmosphere as SO_2 . The data regarding the acidification of soils by these SO_2 emissions is, however, contradictory. Lore (1984) examined soil pH changes over a 9 year period in soils near the SO_2 -emitting Waterton Gas Plant in southern Alberta and found that no acidification trend was occurring; presumably the soil's natural buffering capacity was preventing acidification. This finding does not support the theory of Nyborg et al. (1977) regarding acidification of soils in a 10 to 20 year period of time, nor does it support the work by Laverty and Carson (1977) in which two Alberta soils directly downwind and up to 6 miles from SO_2 plants (16 years in operation) were 0.5 to 1 pH unit lower than soils directly upwind and up to 15 miles from these plants. This latter difference in soil pH may not be significant, however, because seasonal fluctuations in soil pH may vary as much as 2 units (Lore 1984).

The problems of assessing the effects of SO_2 emissions in the acidification of soils are further complicated by the fact that several areas of the province are sulphur deficient. Beaton and Soper (1985) have identified several regions where sulphur deficiencies have occurred, or are most likely to occur, and these include soils which are Black Chernozemic, Grey Luvisolic, and Eutric Brunisols (Figure 7). Nyborg and Walker (1977:174) argue that the SO_2 emissions could supply "at least a part of the 40 million pounds required to supplement soil reserves." The only problem with this is

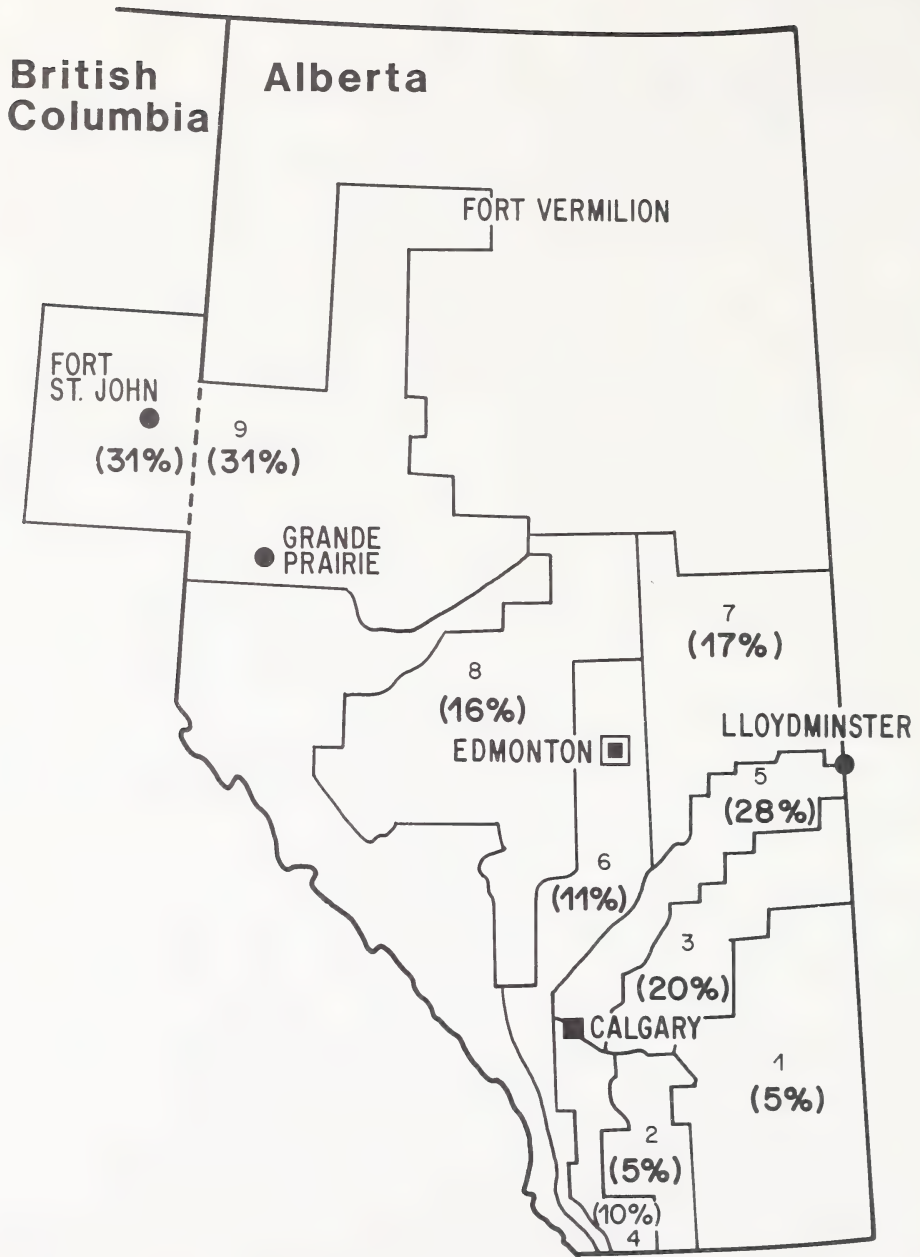
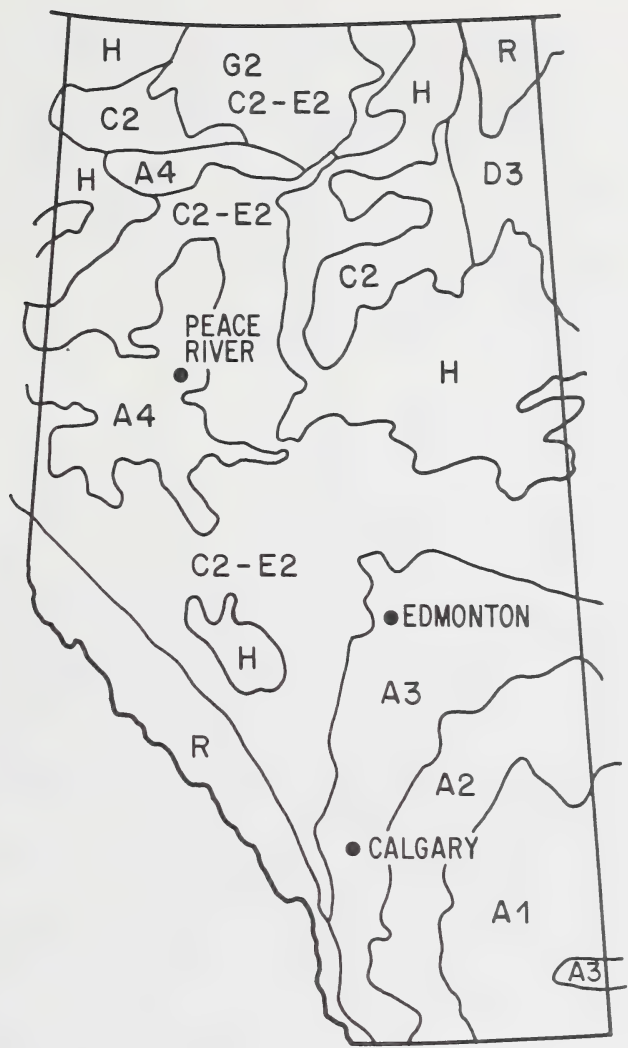


Figure 6. Location of soil testing areas in Alberta, and the percentage of cultivated soil with a pH of 6.0 or less for each area. (Taken from Penney et al. 1977).



- | | |
|---------------------------|--------------------------|
| A1 BROWN CHERNOZEMIC | C2 GREY LUVISOLIC |
| A2 DARK BROWN CHERNOZEMIC | D3 HUMIC FERRIS PODZOLIC |
| A3 BLACK CHERNOZEMIC | H ORGANIC SOILS |
| A4 DARK GREY CHERNOZEMIC | R ROCKLAND |

Figure 7. Soils of Alberta. (From Clayton et al. 1977 in Beaton in American Society of Agronomy Monograph on Sulphur (in press)).

Table 5. Average crop yield as influenced by soil pH and liming.

Crop	pH Range	No. of Sites	Average Yield (kg/ha)			% Increase From Lime
			No Lime	% * Lost	Lime	
Barley	5.0 + less	5	1790	54	3550	98
(Galt)	5.1 - 5.5	17	2960	25	3450	17
	5.6 - 6.0	7	3930		4140	5
Alfalfa	5.0 + less	6	1580	64	6330	301
	5.1 - 5.5	13	3110	28	5300	70
	5.6 - 6.0	8	4350		6420	48

* Percentage of yield lost in unlimed acid soils relative to crop yield in the pH range 5.6 - 6.0.

Source: Penney et al. (1977) and Nyborg and Walker (1977).

that SO_2 deposition decreases drastically as the distance from the source point increases, making equal distribution of the SO_2 an impossibility. Furthermore, getting SO_2 to only those plant species deficient in sulphur could not be controlled. However, the feature of different sulphur requirements for various crops on the soils may explain, in part, certain discrepancies noted by researchers with regard to soil acidification by SO_2 emissions.

Another factor complicating assessments of acidity from SO_2 emissions is that of fertilizer practices on agricultural soil in the province. Ammonium based fertilizers (e.g., ammonium nitrate, ammonium sulphate) are usually converted to nitric acid via the nitrification process (Nyborg and Walker 1977). However, when fertilizer applications are modest, this aspect of creating acid soil would not be considered large.

Although the problems of determining SO_2 contributions to soil acidity are complicated, there are two other concerns associated with the sour gas processing industry; both of these are related to the product sulphur. The first problem deals with the subject of sulphur dust deposition. As background information, this dust can be created in two ways. In one, the recovered elemental sulphur derived from the Claus process is stored by pouring molten sulphur into huge blocks (often the size of buildings and highly visible for several miles). The problem stems from the loose powdered sulphur on the surfaces of these blocks which can often be swept away by winds and deposited on nearby soils. This situation of creating loose powder can often be aggravated by the digging of heavy machinery when loading sulphur on train cars for subsequent shipment and sale. The other way in which sulphur dust is formed is via a S^0 granulation operation which essentially involves melting the sulphur and passing the sulphur over a stream of cool air. A classic example of this operation is provided by the Canterra Energy Limited S^0 granulation operation at the West Whitecourt Gas Plant in Alberta. Legge et al. (1986) have estimated the sulphur deposition within 5.55 km of the S^0 dust emission source to be as low as 374,222 kg and perhaps as high as 748,448 kg. In addition to this sulphur loss, however, is the environmental loss due to the microbial acidification of S^0 to sulphuric acid already described by Laishley (1985) for the above system. Legge et al. (1986) further suggested that the environmental cleanup of these acidified soils would likely be substantial.

Although this latter granulation operation is located in a forest system, there are other numerous sulphur blocks and production plants throughout Alberta and many of these are adjacent to agricultural soils. If these contiguous soils receive continuous input of loose powdered sulphur from these towers in an uncontrolled way, there is little doubt that the sulphur oxidizing microorganisms will convert this sulphur to sulphuric acid, probably dropping the soil pH over time. Even liming such soils in an attempt to neutralize the acid only provides optimum conditions for the less acidophilic thiobacilli to continue the process (Laishley 1985). As long as there is a readily available supply of sulphur the thiobacilli will continue to grow (Laishley 1985). Moreover, in view of the study of Penney et al. (1977) in which a small pH decrease (0.5 to 1.0 unit) affected productivity of crops like alfalfa and barley, one can only conclude that these agricultural soils are at high risk of becoming infertile.

A second problem associated with the sulphur block tower is now centred on recovery of the sulphur block pads on which the towers sit. Since the 1970's the demand for Alberta's sulphur has increased to the extent that most of the surplus sulphur will be exhausted in the not too distant future (J.B. Hyne, per. comm.). Unfortunately, many of the sulphur blocks were laid directly on the bare soil and it is estimated that there may be 20% to 30% sulphur content left in the soil at the end of the cleanup of the blocks (J.B. Hyne, per. comm.). These soils will be subject to intensified microbiological acidification processes, particularly if the sulphur is in the powder form, unless steps are taken to significantly reduce the percentage of sulphur remaining in the soils after the blocks of sulphur are removed.

As we have seen, the problems associated with SO_2 soil acidification are difficult to demonstrate, especially in agricultural soils. However, the soil acidification associated with fugitive sulphur dust is more obvious and, irrespective of soil type or characteristics, the sulphur oxidizing microorganisms will oxidize the sulphur to sulphuric acid. However, the time for acidification of different soils does depend on soil type, soil moisture, soil temperature, and soil buffering capacity (Laishley and Bryant 1985).

6. PREVENTION AND RECLAMATION OF ACID SOILS

6.1 ACID SOILS-RECLAMATION

Reclamation of acid soils is a very difficult process. The main principle in the recovery operation involves neutralization of the acidity followed by restoration of the nutrient status. Neutralization of the acidity has traditionally been accomplished by using lime (CaCO_3). Metson et al. (1977) have estimated that three parts by weight of CaCO_3 will neutralize one part of sulphuric acid.

Reports on the effectiveness of liming indicate that a temporary increase in the mineralization of organic N may be expected (Nyborg and Hoyt 1978) and there may also be increases in the microbial activity of surface material (Ivarson 1977). Although liming generally raises the soil pH quickly, it is not regarded as a panacea for the treatment of all acid soils. Laishley (1985) noted, for example, that liming an acid soil polluted with sulphur only masked an existing problem and really provided a more favourable environment for continued microbial sulphur oxidation, and hence increased acid production. Nyborg (1974) examined another problem associated with liming acid soils. Heavily limed treatments of acid soils containing unreacted elemental S^0 showed severe nitrogen and phosphorous deficiency for plant growth due to increased sulphur oxidizing bacterial activity. It is hard to conceive that this specialized group of bacteria alone could deplete the nitrogen reserve of that soil. Rather, it probably resulted from the combined increase in overall soil microbial activity at neutrality, followed by leaching out of these important inorganic nutrients due to the rapid re-establishment of acid conditions by the thiobacilli. Obviously, reclaiming any acid soil without considering other complicating factors (such as the continued presence of sulphur in the system) may be more disastrous than leaving the soil in its perturbed state. There is therefore a need for extensive microbiological research on liming acid soils and the subsequent effects on soil properties and dynamics.

Assuming that there are no complicating factors from liming acid soil such as those indicated above, it is conceivable that those soils which have experienced acid conditions for long periods are still deficient in nutrients and in the microbial populations so important in nutrient cycle processing.

Suggestions have therefore been made that the recovery of acid soils may be accelerated by transferring these soils to unpolluted sites or by mixing fresh microbiologically active organic soil with the polluted soil (Bewley and Stotzky 1983; Killham and Wainwright 1984). Bewley and Stotzky (1983) observed that such a treatment did improve the mineralization of carbon somewhat in the acid soil. However, Killham and Wainwright (1984) noted that this treatment did not show signs of improving a sulphur polluted soil over a 30 month period, suggesting, as Hoyt and Turner (1975) had noted, that fresh organic amendments to acidified soils may be ineffective or that recovery may be an extremely long process (Coulter 1973).

It should be obvious that reclaiming acid soils is a very serious problem. As noted above, the prospects of reclamation of acid soils are at best cautiously optimistic. Perhaps, though, our efforts should be directed at prevention of acid deposition in the first place. As it has been predicted that the existing stock piles of sulphur are likely to be consumed through sales in the market-place over the next 20 years (Hyne,

pers. comm.), it would seem that the recovery of the last 3% to 5% of fugitive SO₂ from sour gas processing plants is practical. Not only would any environmental problems related to acid deposition from SO₂ be eliminated, but industry would benefit by recovering more saleable sulphur. Although the initial cost of reclaiming the 3% to 5% of the SO₂ might be expensive, the monies would probably be recovered in the long run.

6.2 ACID SOILS - SULPHUR POLLUTION, A SPECIAL CASE

Reclamation of acid soils containing S⁰ poses unique problems. Because of the simple nutritional requirements of the thiobacilli (i.e. energy from sulphur oxidation and carbon from CO₂), treating these soils with lime only does not really accomplish very much, other than deluding one into thinking that the acidity problem has been rectified. As we have pointed out in an earlier discussion, the thiobacilli generally acidify soils through a succession of different species. Adjusting the pH to neutrality merely provides an ideal environment for the less acidophilic thiobacilli to repeat this acidifying process (Laishley 1985). Therefore, to ensure that the acid forming reactions are stopped, one is faced with two simple alternatives, either to reduce the sulphur content in the soil or to develop a new liming procedure.

In considering the reduction of sulphur, one simple means would be to place stringent controls on those industrial prilling operations which produce contaminating wind-blown sulphur dust. Perhaps too, sulphur blocks could be made safer by covering them with a tarpaulin which would cut down on wind-blown sulphur particles - something that would be far cheaper than reclaiming lost farm land for miles around the sulphur blocks.

A more serious problem is encountered in the block pad recovery operation where it is estimated that the residual sulphur in the soil may constitute as much as 30% (Hyne, pers. comm.). Even though Hyne's research group is developing a procedure in which the sulphur content may be reduced to as low as 5%, this sulphur could still pose a problem, especially when one considers that 0.5% sulphur in pure culture experiments can support millions of thiobacilli cells per ml (Laishley, unpublished data).

Reclaiming acid soil is not a simple task. With acid soils generated by microbial oxidation of sulphur, however, there is one advantage and that is regarding an early warning of a potential problem. From the thiobacilli detection method developed by Laishley and Bryant (1985), it is easy to monitor and note when these bacterial populations are increasing. As this event often occurs years before actual soil acidification, one at least has some advanced warning of a future problem. The best correction measure at this point is obvious - eliminate the sulphur.

7. FUTURE WORK

As a result of this literature review a number of areas have been revealed which require further study. These topics are outlined in point form below:

1. Traditionally, CaCO_3 has been used in bringing acid soils back to neutral pH. However, CaCO_3 when neutralizing acid, releases the CO_2 required as a carbon source for growth by the sulphur oxidizing thiobacilli. Perhaps in certain circumstances (i.e., sulphur polluted soils) it would be better to use Ca(OH)_2 in place of CaCO_3 - at least here neutralization of acid would not yield CO_2 . We make this statement in full awareness that others (Nyborg 1982:450) do not recommend Ca(OH)_2 because it "raised pH so high that soils remained barren." We believe that armed with a knowledge of the soil's buffering capacity, a controlled adjustment to any soil pH is attainable and, moreover, that pH values raised too high are a fault of the investigator and not of the neutralizing agent. We know too that CaCO_3 additions to an acid/sulphur soil merely provide the aerobic thiobacilli with virtually an unlimited supply of CO_2 for cellular synthesis and growth (Laishley 1985). We do agree that the pH of the acid soil must be adjusted to 7.0 but in these special acid conditions we feel that Ca(OH)_2 would be a more effective acid neutralizing agent, where no CO_2 is evolved in the neutralizing reaction. Therefore, we recommend a comparative time study on the effectiveness of CaCO_3 versus Ca(OH)_2 in treating acid soils and the effects of these treatments on the thiobacilli and general soil microbial activity.
2. As argued previously in Section 6.1, there appear to be inconsistencies in Nyborg's (1974) reasoning for nitrogen and phosphorous deficiencies (in heavily limed acid soils containing unreacted sulphur) due to thiobacilli uptake of these nutrients for growth. We feel that these losses are due in part to an increased total microbial activity at neutrality followed by leaching processes caused by rapid re-establishment of acid conditions by the thiobacilli. These depicted events represent our best judgement of the situation at the present time and would require further investigation to clarify.

In another related problem it has been clearly shown that the activity of certain physiological groups of microorganisms was seriously reduced in the presence of acid conditions (Bryant et al. 1979). However, there is no information in the literature to indicate what effect liming has on these physiological groups. The question remains as to the time required to re-establish normal soil biological activity. Of course, we have assumed here that given enough time, the soil would return to its natural physiological state. However, this may not necessarily be so as certain physiological groups may be permanently destroyed. There is no doubt that answers to questions on this topic would aid in the formulation of further recommendations for recovery of acid soils.

3. Although we have found species of thiobacilli (one acidophilic and other less acidophilic) with a network of glycocalyx material surrounding the cell wall, it would be useful to see if this was a structure common to all species of Thiobacillus. If this was indeed a common structure, and, hence a common mechanism for attachment of the thiobacilli to sulphur, then one would be in a better position to make recommendations regarding the control of these organisms and their acidification processes in soil.
4. It would be advisable to take advantage of our pioneering TEW (Thiobacillus Early Warning) system and have soils at high risk for sulphur acidification routinely examined for their thiobacilli counts. We have the expertise and facilities for running this biological monitoring program. It should be noted here that if these numbers are high (i.e., greater than 10^2 g⁻¹ dry weight soil) immediate preventative measures should be invoked to stop further acidifying activity. Information from point 3 (above) may provide useful input in this situation.

8. REFERENCES CITED

- Abrahamsen, G., R. Horntvedt, and B. Tveite. 1975. Impacts of acid precipitation on coniferous forest ecosystems. Research Report No. 2. SNSF - Project, 1432. Aas-NLH. Oslo, Norway.
- Adamczyk-Winiarska, Z., M. Krol, and J. Kobus. 1975. Microbial oxidation of elemental sulphur in brown soil. *Plant and Soil* 43: 95-100.
- Aleem, M.I.H. 1969. Generation of reducing power in chemosynthesis. VI. Energy linked reactions in the chemoautotroph Thiobacillus neopolitanus. *Antonie van Leeuwenhoek* 35: 379-391.
- Alexander, M. 1971. *Microbial Ecology*. New York: John Wiley and Sons. 511 pp.
- Attoe, O.J. and R.A. Olson. 1966. Factors affecting rate of oxidation in soils of elemental sulfur and that added in rock phosphate sulfur fusions. *Soil Science* 101: 317-325.
- Baas Becking, L.G.M. 1925. Studies on the sulfur bacteria. *Annals of Botany* 39: 613-650. (Original not seen; taken from Pfennig 1975.)
- Babich, H. and G. Stotzky. 1978a. Atmospheric sulfur compounds and microbes. *Environmental Research* 15: 513-531.
- Babich, H. and G. Stotzky. 1978b. Influence of pH on inhibition of bacteria, fungi and coliphages by bisulfite and sulfite. *Environmental Research* 15: 405-417.
- Bacon, R.F. and R. Fanelli. 1942. Purification of sulphur. *Industrial Engineering Chemistry* 34: 1043-1048.
- Beaton, J.D. and R.J. Soper. 1986. Plant response to sulphur in western Canada. *American Society of Agronomy Monograph* (in press).
- Beudeker, R.F., J.W.M. Kerver, and J.G. Fuenen. 1981. Occurrence, structure, and function of intracellular polyglycose in the obligate chemolithotroph Thiobacillus neopolitanus. *Archives of Microbiology* 19: 221-226.
- Bewley, R.J.F. and G. Stotzky. 1983. Anionic constituents of acid rain and microbial activity in soil. *Soil Biology and Biochemistry* 15: 431-437.
- Bloomfield, C. 1967. Effect of some phosphate fertilizers on the oxidation of elemental sulfur in soil. *Soil Science* 115: 219-223.
- Bloomfield, C. and J.K. Coulter. 1973. Genesis and management of acid sulphate soils. *Advances in Agronomy* 25: 265-326.
- Bollen, W.B. 1977. Sulfur oxidation and respiration in 54 year old soil samples. *Soil Biology and Biochemistry* 9: 405-410.
- Boyd, R.F. 1984. Chapter 13: Energy production in microorganisms. *In*: *General Microbiology*, ed. D.L. Bowen. St. Louis, Missouri: Times Mirror/Mosby College Publishing, pp. 281-311.
- Bremner, J.M. and L.G. Bundy. 1974. Inhibition of nitrification in soils by volatile sulfur compounds. *Soil Biology and Biochemistry* 6: 161-165.
- Brierley, C.L. 1982. Microbiological mining. *Scientific American*, August: 45-53.
- Brierley, C.L. 1978. Bacterial leaching. *CRC Critical Reviews in Microbiology* 6: 207-262.
- Brock, T.D., D.W. Smith, and M.T. Madigan. 1984. *Biology of Microorganisms*. 4th Ed. Englewood Cliffs, New Jersey: Prentice Hall. pp. 704-715.
- Brock, T.D., K.M. Brock, R.T. Belly, and R.L. Weiss. 1972. *Sulfolobus*. A new genus of sulfur oxidizing bacteria living at low pH, and high temperature. *Archiv Mikrobiologia* 84: 54-68.

- Bryant, R.D., E.A. Gordy, and E.J. Laishley. 1979. Effect of soil acidification on the soil microflora. *Water, Air, and Soil Pollution* 11: 437-445.
- Bryant, R.D., E.J. Laishley, C.L. Labine, and J.B. Hyne. 1985a. Thiobacilli and wet sulphur corrosion. *ASR Quarterly Bulletin* XXII: 1-11.
- Bryant, R.D., E.J. Laishley, and J.B. Hyne. 1986. The sulphur-microbe interface. Chalk-talk presented at Alberta Sulphur Research Ltd., Twenty-first Annual General Meeting. 1985 June; The University of Calgary, Calgary, Alberta. (in press)
- Bryant, R.D., J.W. Costerton, and E.J. Laishley. 1984. The role of *Thiobacillus albertis* in the adhesion of cells to elemental sulfur. *Canadian Journal of Microbiology* 30: 81-90.
- Bryant, R.D., K.M. McGroarty, J.W. Costerton, and E.J. Laishley. 1983. Isolation and characterization of a new acidophilic *Thiobacillus* species (*T. albertis*). *Canadian Journal of Microbiology* 29: 1159-1170.
- Buchanan, R.E. and N.E. Gibbons, eds. 1974. *Bergey's Manual of Determinative Bacteriology*, 8th Ed. Williams and Wilkens. Baltimore, Maryland: Waverly Press, pp. 456-461.
- Burton, S.D. and R.Y. Morita. 1964. Effect of catalase and culture conditions on growth of *Beggiatoa*. *Journal of Bacteriology* 88: 1755-1761.
- Cadle, R.D. 1975. The sulfur cycle. In: *Sulfur in the Environment*, ed. H.S. Parker. St. Louis, Missouri: Missouri Botanical Gardens and Union Electric Company, pp. 1-13.
- Cappenberg, Th.E. 1975. Relationships between sulfate-reducing and methane producing bacteria. *Plant and Soil* 43: 125-139.
- Cappenberg, Th.E. 1974. Interrelations between sulfate-reducing and methane producing bacteria in bottom deposits of a freshwater lake. II. Inhibition experiments. *Antonie van Leeuwenhoek Journal of Microbiology and Serology* 40: 297-306.
- Carpenter, P.L. 1977. *Microbiology*, 4th Ed. Philadelphia: W.B. Saunders, p. 121.
- Clayton, J.S., W.A. Ehrlich, D.B. Cann, J.H. Day, and I.B. Marshall. 1977. *Soils of Canada*. Research Branch, Canada Department of Agriculture, Ottawa, Ontario.
- Coleman, R. 1966. The importance of sulphur as a plant nutrient in world crop production. *Soil Science* 101: 230-239.
- Costerton, J.W. and R.T. Irvin. 1981. The bacterial glycocalyx in nature and disease. *Annual Review of Microbiology* 35: 299-324.
- Culter, J.K. 1973. The management of acid sulphate and pseudo acid sulfate soils for agriculture and other uses. In: *Proceedings, International Symposium on Acid Sulphate Soils*, Wageningen 1: 255-273.
- DiSpirito, A.A., M. Silver, L. Voss, and O.H. Tuovinen. 1982. Flagella and pili of iron-oxidizing thiobacilli isolated from a uranium mine in northern Ontario, Canada. *Applied Environmental Microbiology* 43: 1196-1200.
- Dochinger, L.S. and T.A. Seliga. 1975. Acid precipitation and the forest ecosystem. *Journal of the Air Pollution Control Association* 25: 1103-1105.
- Edelman, C.H. and J.M. van Staveren. 1958. Marsh soils in the United States and The Netherlands. *Journal of Soil and Water Conservation* 13: 5-17.
- Francis, A.J. 1982. Effects of acidic precipitation and acidity on soil microbial processes. *Water, Air, and Soil Pollution* 18: 375-394.
- Freney, J.R. 1961. Some observations on the nature of organic compounds in soil. *Australian Journal of Agricultural Research* 12: 424-432.

- Germida, J.J., J.R. Lawrence, and V.V.S.R. Gupta. 1985. Microbial oxidation of sulphur in Saskatchewan soils. *In*: Proceedings of Sulphur - 84, an International Conference on Sulphur, Calgary, Alberta. 1984 June 3-6; Sulphur Development Institute of Canada; pp. 703-710.
- Ghiorse, W.C. and M. Alexander. 1976. Effect of microorganisms on the sorption and fate of sulfur dioxide and nitrogen dioxide in soil. *Journal of Environmental Quality* 5: 227-230.
- Gorham, E. 1976. Acid precipitation and its influence upon aquatic ecosystems - an overview. *Water, Air, and Soil Pollution* 6: 471-481.
- Gottschalk, G. 1979. *Bacterial Metabolism*. New York: Springer Verlag. 281 pp.
- Greenhalgh, J.R. and N.D. Mitchell. 1976. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of Brassica oleracea. *New Phytologist* 77: 391-398.
- Grenffelt, P., C. Bengtson, and L. Skarby. 1980. An estimate of the atmospheric input of acidifying substances to a forest ecosystem. *In*: Effects of Acid Precipitation on Terrestrial Ecosystems, eds. T.C. Hutchinson and M. Havas. New York: Plenum Press, pp. 29-40.
- Guay, R. and M. Silver. 1975. Thiobacillus acidophilus sp. nov; isolation and some physiological characteristics. *Canadian Journal of Microbiology* 21: 281-288.
- Gunnison, A.F. 1981. Sulphite toxicity: A critical review of *in vitro* and *in vivo* data. *Food and Cosmetics Toxicology* 19: 667-682.
- Hamilton, W.A. 1983. Sulphate-reducing bacteria and the offshore oil industry. *Trends in Biotechnology* 1(2): 36-40.
- Happold, F.C. and A. Kay. 1937. CLXVII: The bacterial purification of gas works liquors II - The bacterial oxidation of ammonium thiocyanate. *Biochemistry Journal* 31: 1323-1329.
- Happold, F.C., K.I. Johnstone, H.I. Rogers, and J.B. Youett. 1954. The isolation and characteristics of an organism oxidizing thiocyanate. *Journal of General Microbiology* 10: 261-268.
- Harrison, A.P. Jr. 1982. Genomic and physiological diversity amongst strains of Thiobacillus ferrooxidans and genomic comparison with Thiobacillus thiooxidans. *Archiv Microbiologia* 131: 68-76.
- Hirt, W.E. and J.R. Vestal. 1975. Physical and chemical studies of Thiobacillus ferrooxidans lipopolysaccharides. *Journal of Bacteriology* 123: 642-650.
- Holt, S.C. and I.J. Beveridge. 1982. Electron microscopy: its development and application to microbiology. *Canadian Journal of Microbiology* 28: 1-53.
- Hoyt, P.B. and R.C. Turner. 1975. Effects of organic materials added to very acid soils on pH, aluminum, exchangeable NH₄ and crop yields. *Soil Science* 119: 227-237.
- Hyne, J.B. 1981. Understanding sulphur - the key to the advancement of its technology. *In*: Proceedings of Sulphur-81, An International Conference on Sulphur. Sponsored by Sulphur Development Institute of Canada, Government of Alberta, and the British Sulphur Corporation Limited; Calgary, Alberta; pp. 25-38.
- Hyne, J.B. 1977. Recovered sulphur - a disposal problem. *Alberta Sulphur Research Ltd. Quarterly Bulletin* XIX: 5-24.
- Hyne, J.B. and B.W. Kobryn. 1981. The ageing of solidified sulphur and effects on formed products. *In*: Proceedings of Sulphur-81, An International Conference on Sulphur. Sponsored by Sulphur Development Institute of Canada, Government of Alberta, and the British Sulphur Corporation Limited; Calgary, Alberta; pp. 621-630.

- Ivarson, K.C. 1977. Changes in decomposition rate, microbial population and carbohydrate content of an acid peat bog after liming and reclamation. *Canadian Journal of Soil Science* 7: 129-137.
- Jorgensen, B.B. 1982. Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. *Philosophical Transactions of the Royal Society of London B* 298: 543-561.
- Justin, P. and D.P. Kelly. 1978. Growth kinetics of *Thiobacillus denitrificans* accompanying the transition from aerobic to anaerobic growth. *Journal of General Microbiology* 107: 131-137.
- Kargi, F. 1982. Microbiological coal desulphurization. *Enzyme Microbiology and Technology* 4: 13-19.
- Katayama-Fujimura, Y., I. Kanashima, N. Tsuzaki, and H. Kuraishi. 1984. Physiological characteristics of the facultatively chemolithotrophic *Thiobacillus* species *Thiobacillus delicatus* nom. rev., emend., *Thiobacillus perometabolis* and *Thiobacillus intermedius*. *International Journal of Systematic Bacteriology* 34: 139-144.
- Katayama-Fujimura, Y., N. Tsuzaki, and H. Kuraishi. 1983a. Reidentification of *Thiobacillus perometabolis* ATCC 27793 and *Thiobacillus* sp. Strain A2 with reference to a new species, *Thiobacillus rapidicrescens* sp. nov. *International Journal of Systematic Bacteriology* 33: 532-538.
- Katayama-Fujimura, Y., N. Tsuzaki, and H. Kuraishi. 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. *Journal of General Microbiology* 128: 1599-1611.
- Katayama-Fujimura, Y., Y. Enokizono, T. Kaneko, and H. Duraishi. 1983b. Deoxyribonucleic acid homologies among species of the genus *Thiobacillus*. *Journal of General and Applied Microbiology* 29: 287-295.
- Kellogg, W.W., R.D. Cadle, E.R. Allen, A.L. Lazrus, and E.A. Martell. 1972. The Sulfur Cycle. *Science* 175: 587-596.
- Kelly, D.P. 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. *Philosophical Transactions of the Royal Society of London B* 298: 499-528.
- Keunen, J.G. 1975. Colorless sulfur bacteria and their role in the sulfur cycle. *Plant and Soil* 43: 49-76.
- Keunen, J.G. and H. Veldkamp. 1972. *Thiomicrospira pelophila*. nov. gen., nov. sp., a new obligately chemolithotrophic colorless sulfur bacterium. *Antonie van Leeuwenhoek Journal of Microbiology and Serology* 38: 241-256.
- Keunen, J.G. and R.F. Beudeker. 1982. Microbiology of thiobacilli and other sulphur-oxidizing autotrophs, mixotrophs and heterotrophs. *Philosophical Transactions of the Royal Society of London B* 298: 473-497.
- Killham, K. and M. Wainwright. 1984. Chemical and microbiological changes in soil following exposure to heavy atmospheric pollution. *Environmental Pollution* A33: 121-131.
- King, R.A. and J.D. Miller. 1971. Corrosion by the sulphate-reducing bacteria. *Nature* 233: 491-492.
- Kocur, M., T. Martinec, and M. Mazanec. 1968. Fine structure of *Thiobacillus novellus*. *Journal of General Microbiology* 52: 344-345.
- Korhonen, T.K., E.-L. Nurmiäho, and O. Tuovinen. 1978. Fimbriation in *Thiobacillus* A2. *FEMS Microbiology Letters* 3: 195-198.
- Ladd, T.I. 1982. Heterotrophic activity of epilithic bacteria communities in a lotic ecosystem. Ph.D. Thesis. Calgary, Alberta: University of Calgary. 402 pp.

- Laishley, E.J. 1985. Sulphur bacteria and soil acidification. In: Acidification in a Temperate Forest Ecosystem. The Role of Sulphur Gas Emission and Sulphur Dust. A Final Report Submitted to the Whitecourt Environmental Study Group. R.L. Findlay, Chairman, Amoco Canada Petroleum Co. Ltd., Calgary, Alberta. pp. 177-192.
- Laishley, E.J. 1978. Implications of microbial attack on sulphur bonded construction materials. Alberta Sulphur Research Ltd. Quarterly Bulletin XV: 26-35.
- Laishley, E.J. and H.R. Krouse. 1978. Stable isotope fractionation by Clostridium pasteurianum. 2. Regulation of sulphite reductases by sulfur amino acids and their influence on sulfur isotope fractionation during SO_3^{2-} and SO_4^{2-} reduction. Canadian Journal of Microbiology 24: 716-724.
- Laishley, E.J. and R.D. Bryant. 1985. Long term environmental effects of sulphur and sulphur concrete on the soil ecosystem. In: Proceedings of Sulphur-84, an International Conference on Sulphur, Calgary, Alberta. 1984 June 3-6; Sulphur Institute of Canada; pp. 641-646.
- Laishley, E.J., M.G. Tyler, and H.R. Krouse. 1984. Sulfur isotope fractionation during SO_3^{2-} reduction by different clostridial species. Canadian Journal of Microbiology 30: 841-844.
- Laishley, E.J., M.G. Tyler, and R.G. McCready. 1979. Biological oxidation of sulphur construction materials. In: Vol. 2. Sulphur in Construction, eds. V.M. Malhotra, J.A. Soles, T.A. Wheat, and E.E. Berry. Ottawa: Canada Centre for Mineral and Energy Technology, pp. 171-180.
- Laishley, E.J., M.G. Tyler, and R.G. McCready. 1978. Environmental assessment of soils in contact with sulfur based construction material. In: Environmental Biogeochemistry and Geomicrobiology Vol. 2. The Terrestrial Environment, ed. W.E. Krumbeln. Ann Arbor, Michigan: Ann Arbor Science, pp. 699-705.
- Laishley, E.J., R.D. Bryant, B.W. Kobryn, and J.B. Hyne. 1983. The effect of particle size and molecular composition of elemental sulphur on ease of microbiological oxidation. Alberta Sulphur Research Ltd. Quarterly Bulletin XX(3): 33-50.
- Laverty, D.H. and J.A. Carson. 1977. Comparison of soil pH in farm fields located at various distances from SO_2 sources. In: Proceedings of Alberta Sulphur Gas Research Workshop III, eds. H.S. Sandhu and M. Nyborg. 1977 November 17-18; University of Alberta, Edmonton, Alberta. Research Secretariat, Alberta Environment; pp. 237-245.
- LeGall, J. and J.R. Postgate. 1973. The physiology of the sulfate reducing bacteria. Advances in Microbial Physiology 10: 81-133.
- Legge, A.H., J. Corbin, J. Bogner, M. Strosher, D. Parkinson, H.R. Krouse, E.J. Laishley, M.J. Cavey, C.E. Prescott, R.J.F. Bewley, M. Nosal, H.U. Schellhase, T.C. Weidensaul, and J. Mayo. 1986. Acidification in a temperate forest ecosystem: The role of sulphur gas emissions and sulphur dust. A Final Report Prepared for the Whitecourt Environmental Study Group by Kananaskis Centre for Environmental Research, The University of Calgary, Calgary, Alberta.
- Li, P. and A.C. Caldwell. 1966. The oxidation of elemental sulfur in soil. Proceedings of the Soil Science Society of America 30: 370-372.
- London, J. 1963. Thiobacillus intermedius nov. sp. A novel type of facultative autotroph. Archiv fur Mikrobiologie 46: 328-337.
- Lore, J.A. 1984. Observations of pH in field soils near a sour gas plant in Alberta. Water, Air, and Soil Pollution 21: 403-410.
- Mahoney, R.P. and M.R. Edwards. 1966. Fine structure of Thiobacillus thiooxidans. Journal of Bacteriology 92: 487-495.
- Malmer, N. 1976. Acid precipitation: chemical changes in the soil. Ambio 5: 231-234.

- Matin, A. and M. Matin. 1982. The proton motive force and the ΔpH in spheroplasts of an acidophilic bacterium (Thiobacillus acidophilus). *Journal of General Microbiology* 128: 3071-3075.
- McCready, R.G.L., E.J. Laishley, and H.R. Krouse. 1974. The effect of sulfate and sulfite on growth and sulphur isotope fractionation by Clostridium pasteurianum. Canadian Sulphur Symposium. 1974 May 30-June 1; The University of Calgary, Calgary, Alberta; pp. F1-F7.
- Metson, A.J., D.J. Gibson, J.E. Cos, and D.B. Gibbs. 1977. The problem of acid sulphate soils, with examples from North Auckland, New Zealand. *New Zealand Journal of Science* 20: 371-395.
- Meyer, B. 1976. Elemental sulfur. *Chemical Review* 76: 367-388.
- Moser, U.S. and R.V. Olson. 1953. Sulfur oxidation in four soils as influenced by soil moisture tension and sulfur bacteria. *Soil Science* 76(4): 251-257.
- Nelson, D.C. and R.W. Castenholz. 1981. Use of reduced sulfur compounds by Beggiatoa sp. *Journal of Bacteriology* 147: 140-154.
- Nor, Y.M. and M.A. Tabatabai. 1977. Oxidation of elemental sulfur in soils. *Soil Science Society of America Journal* 41: 736-741.
- Nugent, J.H.A. 1984. Photosynthetic electron transport in plants and bacteria. *Trends in Biochemical Sciences* 9: 354-357.
- Nyborg, M. 1982. Environmental implications of windblown sulphur from sour natural gas processing plants in Alberta. In: Proceedings of the International Sulphur 82 Conference, Vol. I., ed. A.I. More. 1982 November; London: British Sulphur Corp. Ltd.; pp. 445-462.
- Nyborg, M. 1974. Reclamation of soils and waters made acid by windblown sulphur dust. In: Proceedings of a Workshop on Reclamation of Disturbed Lands in Alberta, eds. P. Hocking and W.R. MacDonald. 1974; Department of Environment Research Secretariat, Edmonton, Alberta; pp. 55-71.
- Nyborg, M. and D.R. Walker. 1977. An overview - The effects of sulphur dioxide emission on the acidity and sulphur content of soils. In: Proceedings of Alberta Sulphur Gas Research Workshop III, eds. H.S. Sandhu and M. Nyborg. 1977 November 17-18; University of Alberta, Edmonton, Alberta. Research Secretariat, Alberta Environment; pp. 165-195.
- Nyborg, M. and P.B. Hoyt. 1978. Effects of soil acidity and liming on mineralization of soil nitrogen. *Canadian Journal of Soil Science* 58: 331-338.
- Nyborg, M., J. Crepin, D. Hocking, and J. Baker. 1977. Effect of sulphur dioxide on precipitation and on the sulphur content and acidity of soils in Alberta, Canada. *Water, Air, and Soil Pollution* 7: 439-448.
- Oden, S. 1971. Some effects of the acidity of air and precipitation. Proceedings of International Air Pollution Control and Noise Abatement Exhibition Conference 1: 85-94.
- Overrein, L.N. 1972. Sulphur pollution patterns observed, leaching of calcium in forest soil determined. *Ambio* 1: 145-147.
- Parker, C.D. 1947. Species of sulphur bacteria associated with corrosion of concrete. *Nature* 159: 439-440.
- Parker, C.D. and J. Prisk. 1953. The oxidation of inorganic compounds of sulfur by various sulfur bacteria. *Journal of General Microbiology* 8: 344-364.
- Peck, H.D. 1960. Adenosine 5'-phosphosulfate as an intermediate in the oxidation of thiosulfate by Thiobacillus thioporus. Proceedings of National Academy of Science U.S. 46: 1053-1057.

- Peck, H.D., Jr. 1975. The microbial sulfur cycle in sulfur in the environment. *In: Sulfur in the Environment*, ed. H.S. Parker. St. Louis, Missouri: Missouri Botanical Gardens and Union Electric Company, pp. 62-79.
- Peck, H.D., Jr. 1961. Enzymatic basis for assimilatory and dissimilatory sulfite reduction. *Journal of Bacteriology* 82: 933-939.
- Penney, D.C., M. Nyborg, P.B. Hoyt, W.A. Rice, B. Siemens, and D.H. Lavery. 1977. An Assessment of the soil acidity problem in Alberta and Northwestern British Columbia. *Canadian Journal of Soil Science* 57: 157-164.
- Pfennig, N. 1975. The phototrophic bacteria and their role in the sulfur cycle. *Plant and Soil* 43: 1-6.
- Postgate, J.R. 1982. Economic importance of sulphur bacteria. *Philosophical Transactions of the Royal Society London B* 298: 583-600.
- Postgate, J.R. 1979. *The Sulphate Reducing Bacteria*. Cambridge, England: Cambridge University Press. 151 pp.
- Powlson, D.S. and D.S. Jenkinson. 1971. Inhibition of nitrification in soil by carbon disulfide from rubber bungs. *Soil Biology and Biochemistry* 3: 267-269.
- Reuss, J.O. 1983. Implications of calcium-aluminum exchange system for the effect of acid precipitation on soils. *Journal of Environmental Quality* 12: 591-595.
- Reuss, J.O. 1975. Sulfur in the soil system. *In: Sulfur in the Environment*, ed. H. S. Parker. St. Louis, Missouri: Missouri Botanical Gardens and Union Electric Company, pp. 51-62.
- Reynolds, D.M., E.J. Laishley, and J.W. Costerton. 1981. Physiology and ultrastructural characterization of a new acidophilic *Thiobacillus* species (*T. kabobis*). *Canadian Journal of Microbiology* 27: 151-161.
- Rorison, I.H. 1973. The effect of extreme acidity on the nutrient uptake and physiology of plants. *Proceedings International Symposium on Acid Sulphate Soils, Wageningen* 1: 223-253.
- Ruby, E.G., C.O. Wirsén, and H.W. Janasch. 1981. Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos rift hydrothermal vents. *Applied Environmental Microbiology* 42: 317-324.
- Santer, M., J. Boyer, and V. Santer. 1959. *Thiobacillus novellus*. Growth on organic and inorganic media. *Journal of Bacteriology* 78: 197-202.
- Shively, J.M. 1974. Inclusion bodies of prokaryotes. *Annual Review of Microbiology* 28: 167-187.
- Shively, J.M., G.L. Decker, and J.W. Greenwalt. 1970. Comparative ultrastructure of the *Thiobacilli*. *Journal of Bacteriology* 101: 618-627.
- Silverman, M.P. 1967. Mechanism of bacterial pyrite oxidation. *Journal of Bacteriology* 94: 1046-1051.
- Sorokin, Yu. I. 1970. Interrelations between sulphur and carbon turnover in meromictic lakes. *Archiv Hydrobiologia* 66: 391-466.
- Stanier, R.Y. 1961. Photosynthetic mechanisms in bacteria and plants: development of a unitary concept. *Bacteriology Review* 25: 1-17.
- Starkey, R.L. 1966. Oxidation and reduction of sulfur compounds in soils. *Soil Science* 101(4): 297-306.
- Starkey, R.L. 1935. Isolation of some bacteria which oxidize thiosulfate. *Soil Science* 39: 197-215.

- Stoldal, R. 1982. Homocyclic sulfur molecules. *In*: Topics in Current Chemistry 102, Inorganic Ring Systems, ed. F.L. Boschke. Berlin, Heidelberg, New York: Springer-Verlag, pp. 149-176.
- Stevenson, F.J. 1967. Organic acids in soils. *In*: Soil Biochemistry Volume 1, eds. A.D. McLaren and G.H. Peterson. New York: Marcel Dekker. 509 pp.
- Strohl, W.R. and J.M. Larkin. 1978. Enumeration, isolation and characterization of Beggiatoa from freshwater sediments. Applied Environmental Microbiology 36: 755-770.
- Tabatabai, M.A. and J.M. Bremner. 1972. Forms of sulfur and carbon, nitrogen and sulfur relationships in Iowa soils. Soil Science 114: 380-386.
- Tabatabai, M.A. 1985. Effect of acid rain on soils. CRC Critical Reviews in Environmental Control 15: 65-110.
- Takahashi, K., M. Kasahara, and M. Ito. 1975. A kinetic model of sulfuric acid aerosol formation from photochemical oxidation of sulfur dioxide vapor. Aerosol Science 6: 45-55.
- Takakawa, S., T. Fujimori, and H. Iwasaki. 1979. Some properties of cell-sulfur adhesion in Thiobacillus thiooxidans. Journal of General and Applied Microbiology 25: 21-29.
- Tamm, C.O. 1976. Acid precipitation. Biological effects in soil and on forest vegetation. Ambio 5: 235-238.
- Taylor, B.F. and D.S. Hoare. 1969. New facultative Thiobacillus and a reevaluation of the heterotrophic potential of Thiobacillus novellus. Journal of Bacteriology 100: 487-497.
- Taylor, B.F. and D.S. Hoare. 1971. Thiobacillus denitrificans as an obligate chemolithotroph. II. Cell suspension and enzymatic studies. Archiv Microbiologia 80: 262-276.
- Tobolsky, A.V. and W. J. MacKnight. 1965. Polymeric sulfur and related polymers. Polymer Reviews, Vol 13. New York: Interscience. 140 pp.
- Trudinger, P.A. 1962. The metabolism of inorganic sulphur compounds by Thiobacillus. Review of Pure and Applied Chemistry 17: 1-24.
- Ulrich, B. 1980. Production and consumption of hydrogen ions in the ecosphere. *In*: Effects of Acid Precipitation on Terrestrial Ecosystems, eds. T.C. Hutchinson and M. Havas. New York: Plenum Press, pp. 255-282.
- Wainwright, M. 1980. Man made emissions of sulphur and the soil. International Journal of Environmental Studies 14: 279-288.
- Wainwright, M. 1979. Microbial S-oxidation in soils exposed to heavy atmospheric pollution. Soil Biology and Biochemistry II: 95-98.
- Wainwright, M. 1978. Microbial sulphur oxidation in soil. Science Progress Oxford 65: 459-475.
- Walker, T.W. and A.F.R. Adams. 1958. Studies in soil organic matter. Soil Science 85: 307-318.
- Weir, R.G. 1975. The oxidation of elemental sulphur and sulphides in soil. *In*: Sulphur in Australasian Agriculture, ed. K.D. McLachlan. Sidney, Australia: Sidney University Press, pp. 40-50.
- Woese, C.R. 1981. Archaeobacteria. Scientific American (June): 98-122.
- Wood, T. 1975. Acid precipitation in sulfur in the environment. *In*: Sulfur in the Environment, ed. H. S. Parker. St. Louis, Missouri: Missouri Botanical Gardens and Union Electric Company, pp. 39-50.

U